January 26, 2023

Petition submitted via e-mail and UPS

PETITION TO REQUEST HEALTH AND ENVIRONMENTAL TESTING AND REGULATION ON POLYVINYL ALCOHOL UNDER THE TOXIC SUBSTANCES CONTROL ACT AND AN UPDATE TO THE CHEMICAL SAFETY STATUS OF POLYVINYL ALCOHOL ON THE EPA'S SAFER CHEMICAL INGREDIENTS LISTS

Michael S Regan, Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W. Washington, D.C. 20460
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Dear Administrator Regan,

Please accept the following petition on behalf of Blueland and Plastic Pollution Coalition joined by the following leading 15 nonprofit organizations fighting plastic pollution and climate change: Beyond Plastics, Plastic Oceans International, The Shaw Institute, Lonely Whale, 5 Gyres, GAIA (Global Alliance for Incinerator Alternatives), Oceanic Global Foundation, The Last Beach Cleanup, Rio Grande International Study Center, Inland Ocean Coalition, Occidental Arts and Ecology Center, Turtle Island Restoration Network, Friends of the Earth, Surfrider and Made Safe.

This petition is submitted under section 21 of the Toxic Substances Control Act (TSCA) and the EPA Safer Choice Standards and Safer Chemical Ingredients Lists from the EPA Safer Choice Program. This petition requests that the EPA require human health and environmental health and safety testing for Polyvinyl Alcohol, also known as PVA or PVOH, specifically PVA used in laundry and dishwasher detergent pods and sheets as these are product categories relevant to the EPA Safer Choice program. The petition requests an order under section 4 of TSCA requiring the manufacturers and processors of PVA who are part of the EPA Safer Choice Program, have products with the EPA Safer Choice certification, and who are seeking an EPA Safer Choice certification for pods or sheets products, to fund and conduct this testing under the guidance and direction of independent, third-party scientists. The petition also requests that until such testing is completed and reviewed by the EPA, the EPA update the status of PVA on the EPA Safer Chemical Ingredient List from a "green circle" chemical to a "gray square" to more accurately reflect that more information is needed before human and environmental health impacts can be determined.

Thank you for your consideration.

Signed on behalf of our Blueland, Plastic Pollution Coalition and our co-signed partners.

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Section 1: Executive Summary

This petition is filed under section 21 of the Toxic Substances Control Act (TSCA) and the Safer Choice Standards and Safer Chemical Ingredients Lists from the EPA Safer Choice Program[1]. The petition requests that the Environmental Protection Agency (EPA) require health and environmental safety tests of Polyvinyl Alcohol (PVA, also known as PVOH) used as a plastic film in dishwasher and laundry pods and sheets. The petition requests that the EPA issue an order according to section 4 of the TSCA to require that such testing be funded and conducted by PVA manufacturers and processers who are part of the EPA Safer Choice Program, have products with the EPA Safer Choice certification, and who are seeking an EPA Safer Choice certification for pods or sheets products.[2] This petition also requests that the status of PVA be updated on the Safer Chemical Ingredients List, from a "green circle" to a "gray square" until the EPA can complete a full review of the requested health and environmental safety tests.

PVA is a synthetic, petroleum-derived polymer that can contribute to plastic pollution in oceans, waterways and soil, and recent research suggests that it may negatively impact ecosystems and the food and water supply.[3] PVA also has the potential to exhibit bioaccumulative properties that could carry toxic chemicals and carcinogens up through the food chain.[4] Recent studies have even showed Polyvinyl Alcohol particles to be present in human breastmilk and drinking water.[5] [6]

PVA has many applications, but one fast growing use for PVA, and particular concern for the EPA Safer Choice program, is its use in dishwasher and laundry detergent pods and sheets. Detergent pods are wrapped in a thin layer of PVA plastic film and detergent sheets are woven together with PVA. Laundry and dishwasher pods are a popular format for consumers, with an estimate of over 20 billion PVA wrapped laundry and dishwasher pods used every year in the

[1] 15 U.S.C. 53 §2603. Testing of chemical substances and mixtures http://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim

[2] US EPA, OCSPP. "TSCA Section 4 Test Orders." Overviews and Factsheets, October 9, 2020. https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/tsca-section-4-test-orders.

[3] Rolsky C, Kelkar V. Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate. *International Journal of Environmental Research and Public Health*. 2021; 18(11):6027. https://doi.org/10.3390/ijerph18116027

[4] *Ibid.*

[5] Ragusa, Antonio, Valentina Notarstefano, Alessandro Svelato, Alessia Belloni, Giorgia Gioacchini, Christine Blondeel, Emma Zucchelli, et al. "Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk." *Polymers* 14, no. 13 (June 30, 2022): 2700. https://doi.org/10.3390/polym14132700.

[6] V. C. Shruti et al., "Free, but Not Microplastic-Free, Drinking Water from Outdoor Refill Kiosks: A Challenge and a Wake-up Call for Urban Management," *Environmental Pollution* 309 (September 15, 2022): 119800, https://doi.org/10.1016/j.envpol.2022.119800.

U.S. alone.[7] Regardless, few consumers realize that the PVA film surrounding pods is actually a petroleum-based plastic.[8] For many, once the plastic pod is put into the washing machine or dishwasher, it is out of sight and out of mind; however, there are potential health and environmental implications. Consumers look to initiatives like the EPA Safer Choice program to evaluate whether their products are safe for themselves and the environment.[9] EPA Safer Choice analyzes ingredients, product performance and packaging to ensure that products with an EPA Safer Choice label are safer for individuals and pets, as well as workers health and the environment.[10]

PVA is currently on the Safer Choice Program's Safer Chemical Ingredients List with a green circle, suggesting to consumers that the PVA plastic film encasing laundry and dishwasher pods is safe for people and the environment, and does not have any adverse impacts on the planet. Nevertheless, research shows that ~75% of plastic pods just from laundry and dishwasher detergents remain intact throughout conventional, wastewater treatment, and may persist in our environment, waterways, oceans and soils.[11] PVA is a water-soluble thermoplastic polymer that has the ability to biodegrade in a set of specific conditions and, when used in washing machines and dishwashers, is designed to go down drains.[12] PVA dissolves and is flushed into municipal wastewater but does not fully biodegrade due to the conditions in most wastewater treatment plants (WWTPs).[13] From there, it flows from municipal wastewater into our water systems and soils.[14] For PVA to completely biodegrade, it requires extremely specific conditions to be met in WWTPs, including a particular length of time spent in WWTPs and the presence of certain, acclimated microorganisms needed to degrade PVA completely.[15] If all conditions are not met, intact PVA plastic is released into wastewater effluent which goes into soil, waterways, oceans and beyond. Since these conditions are rarely met, if ever, studies estimate that over ~75% of intact PVA plastic is released back into the environment[16]. Given PVA's potential for persistence, and the unknown impacts it has on the environment, the status of PVA on the EPA Safer Chemical List should be updated from a "green circle" to a "gray square" until extensive health and environmental safety testing can be completed, and PVA's effect on the environment is determined.

Section 2: The Petitioners

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[7] Rolsky C, Kelkar V. *2.*
[8] Rolsky C, Kelkar V. *2.*
[9] US EPA, OCSPP. "Safer Choice Standard and Criteria." Overviews and Factsheets, March 7, 2014. https://www.epa.gov/saferchoice/standard
[10] *Ibid.*
[11] Rolsky C, Kelkar V. 1
[12] Doble, Mukesh, and Anil Kumar. "CHAPTER 9 - Degradation of Polymers." In *Biotreatment of Industrial Effluents*, edited by Mukesh Doble and Anil Kumar, 101–10. Burlington: Butterworth-Heinemann, 2005. https://doi.org/10.1016/B978-075067838-4/50010-5.
[13] *Ibid. 1*
[14] *Ibid. 1*
[15] *Ibid. 1*
[16] *Ibid. 9*

This petition is submitted by Blueland and Plastic Pollution Coalition, and co-signed by 15 nonprofit organizations fighting plastic pollution and climate change: Beyond Plastics, Plastic Oceans International, The Shaw Institute, Lonely Whale, 5 Gyres, GAIA (Global Alliance for Incinerator Alternatives), Oceanic Global Foundation, The Last Beach Cleanup, Rio Grande International Study Center, Inland Ocean Coalition, Occidental Arts and Ecology Center, Turtle Island Restoration Network, Friends of the Earth, Surfrider and Made Safe. Blueland is a consumer-packaged goods company that aims to eliminate single-use plastic packaging from everyday products, including cleaning and personal care products. Plastic Pollution Coalition is a non-profit communications and advocacy organization that collaborates with an expansive global alliance of organizations, businesses, and individuals to create a more just and equitable world free of plastic pollution and its toxic impacts. Beyond Plastics, Plastic Oceans International, The Shaw Institute, Lonely Whale, GAIA (Global Alliance for Incinerator Alternatives), Oceanic Global Foundation, The Last Beach Cleanup, Rio Grande International Study Center, Inland Ocean Coalition, Occidental Arts and Ecology Center, Turtle Island Restoration Network, Friends of the Earth and 5 Gyres are non-profit organizations dedicated to eliminating plastic pollution and protecting the planet from the harms of plastic and other destructive activities on the environment. Made Safe is a third-party certification organization that aims to help businesses achieve high standards in their practices and with their products as it relates to human and environmental health.

Section 3: Relevant Background Information on Plastic Pollution

In the last 30 years, plastic consumption has quadrupled.[17] In 2019, 2.6 million tons of plastic waste ended up in oceans and waterways.[18] Studies show by 2050, there will be more plastic, by weight, in the ocean than fish.[19] Plastic pollution can inflict substantial harm to aquatic and marine environments – globally, 800 species of animal are impacted by marine debris, 100% of marine turtle species have been found to ingest plastic pieces along with 59% of whale, 36% of seal and 40% of seabird species examined.[20]

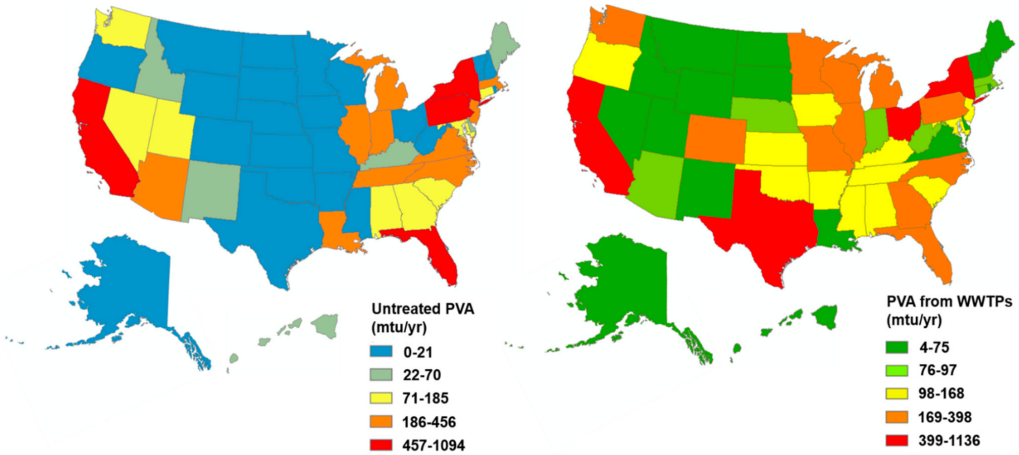
Microplastics are tiny pieces of plastic debris. There is no universally agreed upon size definition for microplastics, though many use plastics less than 5mm in diameter as the guidance.[21] Microplastics have been linked to environmental and human health concerns.[22] Recently,

[17] "Plastic Pollution Is Growing Relentlessly as Waste Management and Recycling Fall Short, Says OECD." Accessed July 21, 2022. https://www.oecd.org/newsroom/plastic-pollution-is-growing-relentlessly-as-waste-management-and-recycling-fall-short.htm.
[18] *Ibid*
[19] *Ibid.*
[20] MBRCtheocean. "Shocking Plastic Statistics." Accessed July 21, 2022. https://www.mbrctheocean.com/pages/shocking-plastic-statistics.
[21] US Department of Commerce, National Oceanic and Atmospheric Administration. "What Are Microplastics?" Accessed July 22, 2022. https://oceanservice.noaa.gov/facts/microplastics.html.
[22] *Ibid.*

microplastics have been found in the human bloodstream and even in human placentas and breastmilk.[23] These tiny pieces of plastic, as well as nanoplastics (which are even smaller), are recognized as a harmful ocean contaminant.[24] Due to their tiny size, they are easily ingested by marine animals, and they also have been found in human food and water sources.[25]

Section 4: PVA Treatment, Pollution and Potential Harms on The Environment

PVA is a synthetic, petroleum-based plastic polymer found in many, everyday products and has become a popular plastic used in cleaning and personal care products.[26] PVA is often found in household items as a thin plastic wrapping, encasing single-dose detergents or woven into laundry detergent sheets. PVA is also increasingly being used in other cleaning and personal care products, such as body washes and toilet cleaners, and is designed to go down drains and into water systems. A recent study published in the *International Journal of Environmental Research and Public Health* titled, "Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate", shows that while PVA does solubilize, it does not necessarily biodegrade. In the U.S., an estimated 61% of PVA goes to WWTPs, and an estimated 37% remains untreated. In total, Rolsky and Kelkar estimate that ~75% of PVA from dishwasher and laundry pods persists through conventional wastewater treatment, passing into waterways and ecosystems beyond.[27] In addition to environmental persistence, similar to other petroleum-based plastic, PVA could bioaccumulate and has the potential to absorb dangerous contaminants and move them up the food chain. This requires further research.[28]



[23] Ragusa, Antonio, Alessandro Svelato, Criselda Santacroce, Piera Catalano, Valentina Notarstefano, Oliana Carnevali, Fabrizio Papa, et al. "Plasticenta: First Evidence of Microplastics in Human Placenta." *Environment International* 146 (January 2021): 106274. https://doi.org/10.1016/j.envint.2020.106274.
[24] Ibid.
[25] Lim, XiaoZhi. "Microplastics Are Everywhere — but Are They Harmful?" *Nature* 593, no. 7857 (May 4, 2021): 22–25. https://doi.org/10.1038/d41586-021-01143-3.
[26] Rolsky and Kelkar, "Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate." 2
[27] *Ibid. 1*
[28] *Ibid. 12.*

Figure 3. from Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate showing the amount of treated and untreated PVA released into the environment in the U.S. in metric ton unit/ year

PVA Degradability Process

PVA from laundry and dishwasher pods and sheets goes from consumer households to WWTPs. Dissolved PVA enters WWTPs but ~75% exits WWTPs intact, which is thought to pollute waterways and soil beyond.[29] This estimate is based on a conventional WWTP process. Facilities with advanced treatment processes may have different degradation rates. PVA goes through the following phases in conventional wastewater treatment plants:

Phase 1: Primary treatment:
In the primary treatment phase, large solids are separated from the wastewater that has entered the facility. In this phase, dissolved PVA is not typically separated from the wastewater due to its hydrophilic properties.[30]

Phase 2: Secondary treatment
If PVA were to biodegrade, it would likely take place in the secondary treatment phase. During the secondary treatment phase, PVA interacts with bacteria and microbes that typically break down biological waste in the wastewater. In conventional WWTPs within the U.S., specific PVA-adapted bacteria and microbes are needed to aid in the near to complete degradation of PVA, though they are not likely present.[31]

If those microbes are present, the PVA in wastewater rarely encounters the microbes for long enough for the PVA to fully degrade. It is estimated that PVA needs to interact with the proper microbes for seven days, and typically wastewater remains in a conventional WWTP for two to three days.[32]

Phase 3: Tertiary treatment

Once the wastewater has gone through the secondary treatment phase, the final phases are disinfection and filtration. In the disinfection phase, it is possible for remaining PVA to be degraded.[33] However, the technology to execute that degradation is expensive, and hence assumed to be rarely employed in WWTPs within the U.S.[34]

[29] *Ibid.*
[30] *Ibid*
[31] *Ibid.*
[32] *Ibid*
[33] *Ibid*
[34] *Ibid.*

After the three treatment phases, wastewater is released from WWTPs. According to the study, PVA can travel into the environment via effluent or released within biosolids. If released with water, it has the potential to impact our waterways and environment. If released within biosolids, it will travel into landfills, onto soils or be incinerated.[35]

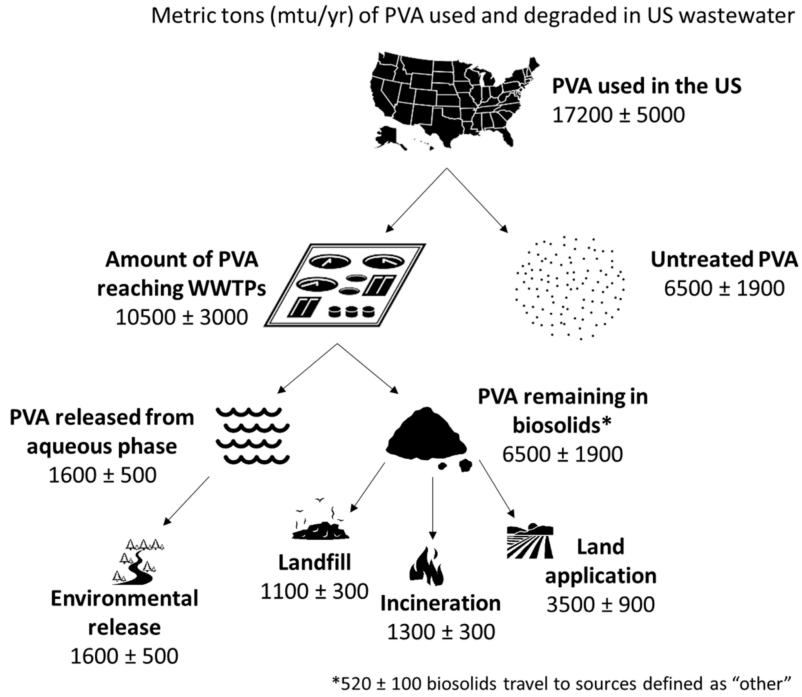


Figure 4 from Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate showing the path of PVA

The ~75% of PVA that remains intact after it leaves the WWTP weighs roughly eight thousand metric tons and is released back into the environment each year, just from plastic laundry and dishwasher pods alone.[36] With PVA's numerous and increasing applications across the cleaning and personal care space, PVA is referred to as one of the most ubiquitous pollutants in waterways and soil.

PVA has various potential impacts on the soil and waterways it pollutes. Further research is still needed to determine the potential harms of PVA in the environment; however, PVA could have the following impacts:

[35] *Ibid.*
[36] Oceans, Plastic. "Detergent Pods Contributing to Plastic Pollution." *Plastic Oceans International* (blog), July 23, 2021. https://plasticoceans.org/detergent-pods-contributing-to-plastic-pollution/.

PVA in soil: PVA that is released from WWTPs as sludge often ends up on land and in soil. This has the potential to affect agricultural yields.[37]

PVA in aquatic environments: A study shows that PVA can alter gas exchanges within aquatic environments. In addition, PVA has exhibited bioaccumulation properties; it has

> "even been documented to mobilize heavy metals from sediments to water resources. Hydrophilic compounds, such as biocides, insecticides, herbicides, flame retardants, corrosion inhibitors, personal care products, and pharmaceuticals are present in wastewater and stormwater. Some of these are proven carcinogens with great aqueous phase stability. As the sorption of organic and inorganic pollutants is not limited to hydrophobic compounds but can also occur with hydrophilic compounds, PVA could act as a vector for transport up the food chain, similarly to more conventional plastics."[38]

Section 5: Current Research on PVA Biodegradability and its Deficiencies

PVA as it's used in dishwasher and laundry pods and sheets has been tested to determine biodegradability, and companies that use PVA utilize these tests to defend their use of the material. These tests evaluate the biodegradability of PVA, typically in laboratories, under the most optimal circumstances. In real world scenarios within conventional WWTPs, neither the conditions in the lab nor the amount of time needed for PVA to fully biodegrade are likely to be met. Many of the tests used to determine PVA's biodegradability rely on OECD standards for biodegradability. While OECD biodegradability standards can be an important tool to determine a material's end of life implications, in the case of PVA and current conditions within WWTPs, these tests are insufficient. The following "readily biodegradability" tests and standards have been used by pods and sheets manufactures and processors to justify their use of PVA for detergent pods and sheets.

> **OECD 301 Test Series to determine "readily biodegradability**": "This Test Guideline describes six methods that permit the screening of chemicals for ready biodegradability in an aerobic aqueous medium. The methods are: the DOC Die-Away, the CO2 Evolution (Modified Sturm Test), the MITI (I) (Ministry of International Trade and Industry, Japan), the Closed Bottle, the Modified OECD Screening and the Manometric Respirometry." [39]
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> "A solution, or suspension, of the test substance, well determined/described, in a mineral medium is inoculated and incubated under aerobic conditions in the dark or in diffuse

[37] *Ibid. 12*
[38] *Ibid. 12*
[39] OECD. *Test No. 301: Ready Biodegradability*. Paris: Organisation for Economic Co-operation and Development, 1992. https://www.oecd-ilibrary.org/environment/test-no-301-ready-biodegradability_9789264070349-en.

light. The running parallel blanks with inoculum but without test substance permits to determine the endogenous activity of the inoculum. A reference compound (aniline, sodium acetate or sodium benzoate) is run in parallel to check the operation of the procedures. Normally, the test lasts for 28 days. At least two flasks or vessels containing the test substance plus inoculum, and at least two flasks or vessels containing inoculum only should be used; single vessels are sufficient for the reference compound. In general, degradation is followed by the determination of parameters such as DOC, CO2 production and oxygen uptake. The pass levels for ready biodegradability are 70% removal of DOC and 60% of ThOD or ThCO2 production for respirometric methods. These pass values have to be reached in a 10-d window within the 28-d period of the test."[40]

OECD 310: "This Test Guideline is a screening method for the evaluation of ready biodegradability of chemicals. The test substance, normally at 20 mg C/L, as the sole source of carbon and energy, is incubated (during 28 days normally) in sealed bottles with aerobic condition containing a buffer-mineral salts medium, which has been inoculated with a mixed population of micro-organisms. In order to check the test procedure, a reference substance (aniline, sodium benzoate or ethylene glycol and 1-octanol) of known biodegradability should be tested in parallel. It is recommended that triplicate bottles be analysed after a sufficient number of time intervals. Also at least five test bottles (from test vessels, blank controls, and vessels with the reference substance) are analysed at the end of the test, to enable 95% confidence intervals to be calculated for the mean percentage biodegradation value. The CO2 evolution resulting from the ultimate aerobic biodegradation of the test substance is determined by measuring the Inorganic Carbon (IC) produced in the test bottles in excess of that produced in blank vessels containing inoculated medium only. The extent of biodegradation is expressed as a percentage of the theoretical maximum IC production (ThIC), based on the quantity of test substance added initially. Biodegradation >60% ThIC within the 10-d window in this test demonstrates that the test substance is readily biodegradable under aerobic conditions."[41]

All six tests in the OECD 301 series and the OECD 310 tests evaluate PVA biodegradability mostly in ex-situ conditions. In these settings, PVA is oftentimes exposed to specific PVA-adapted microorganisms, designed to help break down PVA. In addition, these tests are run for 28 days at a time, and to "pass" the OECD standards, the relevant passing value (different depending on the test) must be reached within a 10-d window.

Research that uses the OECD testing procedures mentioned above to justify PVA's biodegradability fails to address critical gaps between the OECD tests and real-world WWTP conditions. First, OECD test methods can only evaluate PVA in a lab setting. Even if studies use sludge from WWTPs to try to mimic WWTP conditions, testing must aim to accurately match

[40] Ibid.
[41] "Test No. 310: Ready Biodegradability - CO2 in Sealed Vessels (Headspace Test) | READ Online," oecd-ilibrary.org, accessed January 18, 2023, https://read.oecd-ilibrary.org/environment/test-no-310-ready-biodegradability-co2-in-sealed-vessels-headspace-test_9789264224506-en.

WWTP environments in terms of microorganism food-to-microorganism ratio, duration of exposure, etc. In real WWTPs, PVA and other organic and inorganic materials are passing through treatment phases as the same time, so microorganisms present must break down many different materials at once, making microorganism breakdown of PVA less efficient.

Second, this research ignores the 10-day testing window, stating that it does not apply to PVA/PVOH that is used in laundry detergent pods and sheets, because it is not "pure" PVA, despite the OECD stated guidelines.[42] In one study, PVA does not pass the OECD 301B threshold to be readily biodegradable until nearly day 20 of the test[43]. Furthermore, PVA that passes through a wastewater treatment facility typically does so within a 2 or 3-day window, making even the 10-day standard to pass the OECD test insufficient for a substantial biodegradation.

Lastly, many of the OECD standards and test methods (notably much of the 301 series) have not been updated since 1992. Significant advances in testing methods and procedures have been made since these guidelines were first implemented.

Section 6: Requested Actions:

Request for Health and Environmental Safety Tests under the Toxic Substances Control Act

Given the potential for PVA to persist in the environment as a harmful plastic pollutant, this petition requests that the EPA require health and environmental safety tests under the Toxic Substances Control Act on PVA and ultimately regulate PVA used in dishwasher and laundry pods and sheets as a toxic substance, pending the results from testing. The Toxic Substances Control Act seeks to protect human health and the environment by empowering the EPA to issue testing requirements for specific chemicals and establish regulations that restrict manufacturing, processing and distribution of chemicals that are determined a health or environmental risk.[44]

The Toxic Substances Control Act from 15 US Code Chapter 53 Subchapter I section 2603 states:

> "If the finds that—
>
> **(A)(i) (I)**the manufacture, distribution in commerce, processing, use, or disposal of a chemical substance or mixture, or that any combination of such activities, may present an unreasonable risk of injury to health or the environment,

[42] Menzies, Jennifer, Ashley Wilcox, Kenneth Casteel, and Kathleen McDonough. "Water Soluble Polymer Biodegradation Evaluation Using Standard and Experimental Methods." *Science of The Total Environment* 858 (February 1, 2023): 160006. https://doi.org/10.1016/j.scitotenv.2022.160006.

[43] Ibid.

[44] US EPA, "Toxic Substances Control Act (TSCA) and Federal Facilities."

(II)there is insufficient information and experience upon which the effects of such manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted, and

(III)testing of such substance or mixture with respect to such effects is necessary to develop such information; or

(ii)(I)a chemical substance or mixture is or will be produced in substantial quantities, and (aa) it enters or may reasonably be anticipated to enter the environment in substantial quantities or (bb) there is or may be significant or substantial human exposure to such substance or mixture,

(II)there is insufficient information and experience upon which the effects of the manufac

ture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted, and

(III)testing of such substance or mixture with respect to such effects is necessary to develop such information; and

(B) in the case of a mixture, the effects which the mixture's manufacture, distribution in commerce, processing, use, or disposal or any combination of such activities may have on health or the environment may not be reasonably and more efficiently determined or predicted by testing the chemical substances which comprise the mixture;

the Administrator shall by rule, or, in the case of a chemical substance or mixture described in subparagraph (A)(i), by rule, order, or consent agreement, require that testing be conducted on such substance or mixture to develop information with respect to the health and environmental effects for which there is an insufficiency of information and experience and which is relevant to a determination that the manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture, or that any combination of such activities, does or does not present an unreasonable risk of injury to health or the environment.[45]"

Due to the unknown dangers that PVA poses to the environment, the EPA has an obligation, under the TSCA, to require testing of PVA and its end of life in marine and aquatic ecosystems, as well as soil environments, to determine the implications for human and environmental health. This petition requests that the EPA issue an order to require PVA pods and sheets manufacturers and processors who are part of the EPA Safer Choice Program, who have products with the EPA Safer

[45]15 U.S.C. 53 §2603. Testing of chemical substances and mixtures

Choice certification, and who are seeking an EPA Safer Choice certification for pods or sheets products, to fund and conduct full environmental and human health tests on both untreated and treated PVA that is released into aquatic, marine and land environments, under Section 4 of the TSCA. We request that these tests are conducted by and under the direction of independent, third-party labs and are reviewed by the EPA.

Request to Update the Status of PVA on the Safer Chemical Ingredients List

In addition to requesting that PVA be tested and ultimately regulated under the TSCA, this petition requests that the status of PVA be updated from a "green circle" to a "gray square" on the Safer Chemical Ingredients Lists until the requested testing is complete.

The EPA Safer Choice Program has the following guidance on polymers:

> "To be acceptable for labeled products, polymers must have low-concern characteristics. Also, the requirements of this section apply to the low molecular weight components of polymers (typically less than 1,000 daltons). Safer Choice encourages the use of degradable polymers whenever possible; only those that do not degrade into CMRs or PBTs (Persistence, Bioaccumulation, Toxins) will be allowed."[46]

If a polymer does break down into PBTs, it should be excluded from the EPA Safer Chemical list. Using the definitions that the Safer Choice programs outline for persistence and bioaccumulation, PVA should be excluded from the EPA Safer Chemical list.

1. Persistence: "The length of time the chemical can exist in the environment before being destroyed (i.e., transformed) by natural processes".[47]

Rolsky and Kelkar's paper demonstrates that there is significant potential for PVA to persist within waterways, oceans and soils after it leaves WWTPs, with ~75% remaining intact post water treatment.[48]

2. Bioaccumulation: "is a process in which a chemical substance is absorbed in an organism by all routes of exposure as occurs in the natural environment, e.g., dietary and ambient environment sources. Bioaccumulation is the net result of competing processes of chemical uptake into the organism at the respiratory surface and from the diet and chemical

[46] "EPA's Safer Choice Criteria for Colorants, Polymers, Preservatives, and Related Chemicals," n.d., 3.
[47] EPA PBT Final Rule [9]
[48] Rolsky and Kelkar, "Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate." 12

elimination from the organism including respiratory exchange, fecal egestion, metabolic biotransformation of the parent compound and growth dilution".[49]

Rolsky and Kelkar cite Chiellini et. al to suggest that PVA has the potential to exhibit bioaccumulation properties, citing its ability to "mobilize heavy metals from sediments to water resources. Hydrophilic compounds, such as biocides, insecticides, herbicides, flame retardants, corrosion inhibitors, personal care products, and pharmaceuticals are present in wastewater and stormwater".[50]

PVA clearly demonstrates the EPA definitions of Persistence and has the potential for Bioaccumulation. Partially degraded PVA might have the potential to interact with or carry PBT chemicals and bioaccumulate them up the food chain. Because of these characteristics, PVA should not be eligible for the polymer exclusion and should be updated to a "gray square" from the EPA Safer Choice List unless it can be proven to be safe.

Lastly, PVA is used in all laundry and dishwasher pods and sheets. Many brands who use PVA market their pods and sheets as "100% biodegradable" and or "100% plastic-free". Both claims are misleading, given that they use PVA, which is a petroleum-based plastic, and is only biodegradable under extremely specific conditions, as the research by Rolsky and Kelkar explains. These can mislead consumers to think these products are better for the environment than they are, when there is still further research needed. Even with further research, claims such as "100% biodegradable" and "100% plastic-free" would not be substantiated. We request that the EPA Safer Choice Program review claims about PVA through the lens of truth in advertising to ensure that consumers have accurate information about PVA and its potential environmental impacts.

Section 7: Conclusion

PVA plastic film is widely used in conventional household and personal care products. It is designed to become a solution when exposed to water before it is flushed down the drain. PVA can be biodegradable in WWTPs when it encounters a set of extremely specific conditions, as cited above. However, as Rolsky and Kelkar found, these conditions are rarely met in the U.S. Instead, ~75% of PVA from laundry and dishwasher pods likely persists in waterways, soil and oceans.[51] Further research is needed to determine the potential hazards that polluted PVA can pose to ecosystems and human health. Given the gravity of the plastic pollution problem and the known impacts of other plastic polymers on the environment, this petition requests the EPA use its authority under TSCA sections 4 and 21 to require PVA pods and sheets manufacturers and

[49] EPA's Safer Choice Program Master Criteria for Safer Ingredients
[50] Chiellini, Emo, Andrea Corti, Salvatore D'Antone, and Roberto Solaro. "Biodegradation of Poly (Vinyl Alcohol) Based Materials." *Progress in Polymer Science* 28, no. 6 (June 1, 2003): 963–1014. https://doi.org/10.1016/S0079-6700(02)00149-1.
[51] Rolsky and Kelkar, "Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate." 1.

processors who are part of the EPA Safer Choice Program, have products with the EPA Safer Choice certification, and who are seeking an EPA Safer Choice certification for pods or sheets products, to fund independent, third-party extensive health and environmental safety testing of PVA once it is released into ecosystems by way of WWTPs, to determine potential environmental impact. In addition, given the persistence of PVA in the environment and its potential for bioaccumulation, this petition requests immediate update of the status of PVA from a "green circle" to a "gray square" on the EPA Safer Chemical Ingredients Lists until required testing is conducted. Using the polymer exemption definition, the EPA puts forth, a polymer cannot be exempt if it degrades into PBTs (Persistence, Bioaccumulation, Toxins).[52] Rolsky and Kelkar model that PVA likely persists in our environment and Chiellini et al. have shown the potential for PVA to have bioaccumulation properties.[53] For these reasons, it is requested that PVA undergo the requisite testing to determine its impact on the environment and be updated on the Safer Chemical List until the tests funded and submitted by PVA pods and sheets manufacturers and processors who are part of the EPA Safer Choice Program are complete.

[52] EPA PBT Final Rule [9]
[53] Chiellini et al., "Biodegradation of Poly (Vinyl Alcohol) Based Materials."

References:

"15 USC Ch. 53: TOXIC SUBSTANCES CONTROL." Accessed July 21, 2022. http://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim.

Chiellini, Emo, Andrea Corti, Salvatore D'Antone, and Roberto Solaro. "Biodegradation of Poly (Vinyl Alcohol) Based Materials." *Progress in Polymer Science* 28, no. 6 (June 1, 2003): 963–1014. https://doi.org/10.1016/S0079-6700(02)00149-1.

Doble, Mukesh, and Anil Kumar. "CHAPTER 9 - Degradation of Polymers." In *Biotreatment of Industrial Effluents*, edited by Mukesh Doble and Anil Kumar, 101–10. Burlington: Butterworth-Heinemann, 2005. https://doi.org/10.1016/B978-075067838-4/50010-5.

"EPA's Safer Choice Criteria for Colorants, Polymers, Preservatives, and Related Chemicals," n.d., 3.

Lim, XiaoZhi. "Microplastics Are Everywhere — but Are They Harmful?" *Nature* 593, no. 7857 (May 4, 2021): 22–25. https://doi.org/10.1038/d41586-021-01143-3.

Menzies, Jennifer, Ashley Wilcox, Kenneth Casteel, and Kathleen McDonough. "Water Soluble Polymer Biodegradation Evaluation Using Standard and Experimental Methods." *Science of The Total Environment* 858 (February 1, 2023): 160006. https://doi.org/10.1016/j.scitotenv.2022.160006.

Oceans, Plastic. "Detergent Pods Contributing to Plastic Pollution." *Plastic Oceans International* (blog), July 23, 2021. https://plasticoceans.org/detergent-pods-contributing-to-plastic-pollution/.

OECD. *Test No. 301: Ready Biodegradability*. Paris: Organisation for Economic Co-operation and Development, 1992. https://www.oecd-ilibrary.org/environment/test-no-301-ready-biodegradability_9789264070349-en.

"Plastic Pollution Is Growing Relentlessly as Waste Management and Recycling Fall Short, Says OECD." Accessed July 21, 2022. https://www.oecd.org/newsroom/plastic-pollution-is-growing-relentlessly-as-waste-management-and-recycling-fall-short.htm.

Ragusa, Antonio, Valentina Notarstefano, Alessandro Svelato, Alessia Belloni, Giorgia Gioacchini, Christine Blondeel, Emma Zucchelli, et al. "Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk." *Polymers* 14, no. 13 (June 30, 2022): 2700. https://doi.org/10.3390/polym14132700.

Ragusa, Antonio, Alessandro Svelato, Criselda Santacroce, Piera Catalano, Valentina Notarstefano, Oliana Carnevali, Fabrizio Papa, et al. "Plasticenta: First Evidence of Microplastics in Human Placenta." *Environment International* 146 (January 2021): 106274. https://doi.org/10.1016/j.envint.2020.106274.

Rolsky, Charles, and Varun Kelkar. "Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate." *International Journal of Environmental Research and Public Health* 18, no. 11 (January 2021): 6027. https://doi.org/10.3390/ijerph18116027.

Shruti, V. C., Gurusamy Kutralam-Muniasamy, Fermín Pérez-Guevara, Priyadarsi D. Roy, and I. Elizalde-Martínez. "Free, but Not Microplastic-Free, Drinking Water from Outdoor Refill Kiosks: A Challenge and a Wake-up Call for Urban Management." *Environmental Pollution* 309 (September 15, 2022): 119800. https://doi.org/10.1016/j.envpol.2022.119800.

"Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate." *International Journal of Environmental Research and Public Health* 18, no. 11 (January 2021): 6027. https://doi.org/10.3390/ijerph18116027.

MBRCtheocean. "Shocking Plastic Statistics." Accessed July 21, 2022. https://www.mbrctheocean.com/pages/shocking-plastic-statistics.

oecd-ilibrary.org. "Test No. 310: Ready Biodegradability - CO2 in Sealed Vessels (Headspace Test) | READ Online." Accessed January 18, 2023. https://read.oecd-ilibrary.org/environment/test-no-310-ready-biodegradability-co2-in-sealed-vessels-headspace-test_9789264224506-en.

US Department of Commerce, National Oceanic and Atmospheric Administration. "What Are Microplastics?" Accessed July 22, 2022. https://oceanservice.noaa.gov/facts/microplastics.html.

US EPA, OCSPP. "Safer Choice Standard and Criteria." Overviews and Factsheets, March 7, 2014. https://www.epa.gov/saferchoice/standard.

"TSCA Section 4 Test Orders." Overviews and Factsheets, October 9, 2020. https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/tsca-section-4-test-orders.

US EPA, OECA. "Toxic Substances Control Act (TSCA) and Federal Facilities." Other Policies and Guidance, September 19, 2013. https://www.epa.gov/enforcement/toxic-substances-control-act-tsca-and-federal-facilities.

Vitali, Clementina, Ruud Peters, Hans-Gerd Janssen, and Michel W.F.Nielen. "Microplastics and Nanoplastics in Food, Water, and Beverages; Part I. Occurrence." *TrAC Trends in Analytical Chemistry*, May 10, 2022, 116670. https://doi.org/10.1016/j.trac.2022.116670.

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Communication

Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate

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Abstract: Polyvinyl alcohol (PVA) is a water-soluble plastic commercially used in laundry and dish detergent pods (LDPs) for which a complete understanding of its fate in the environment and subsequent consequences is lacking. The objective of this study was to estimate the US nationwide emissions of PVA resulting from domestic use of LDPs, corroborated by a nationwide, online consumer survey and a literature review of its fate within conventional wastewater treatment plants (WWTPs). Peer-reviewed publications focusing on the degradation of PVA in critical processes of WWTPs were shortlisted as a part of the literature review, and subsequent degradation data was extracted and applied to a model with a set of assumptions. Survey and model results estimated that approximately 17,200 ± 5000 metric ton units per year (mtu/yr) of PVA are used from LDPs in the US, with 10,500 ± 3000 mtu/yr reaching WWTPs. Literature review data, when incorporated into our model, resulted in ~61% of PVA ending up in the environment via the sludge route and ~15.7% via the aqueous phase. PVA presence in the environment, regardless of its matrix, is a threat to the ecosystem due to the potential mobilization of heavy metals and other hydrophilic contaminants.

Keywords: biodegradation; dish detergent; laundry; mass loads; microbes; polyvinyl alcohol; wastewater

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1. Introduction

Plastic pollution has been steadily increasing since the 1950s [1]. Due to an upsurge in public awareness regarding plastic usage and pollution, more "sustainable" alternatives have increased in popularity, and are thus being utilized in higher quantities by the general public [2]. These new materials are often marketed as "biodegradable", as they are considered to be susceptible to microbial degradation under specific conditions, but this specificity often makes it difficult to understand their ultimate fate in the environment. Polyvinyl alcohol and its corresponding blends (PVOH, PVAI, Polyviol, Alcotex, Covol, Gelvatol, Lemol, Mowiol, Mowiflex, and Rhodoviol) are examples of polymers that have become more popular both in usage and within scientific research (see Figure 1A) due to their water-solubility. Typically, PVA is used as a protective film for laundry and dish detergents; as a sizing and finishing agent in the textile industry [3]; and as a thickening or coating agent for paints, glues, meat packaging, and pharmaceuticals in paper and food industries (see Figure 1A) [3].

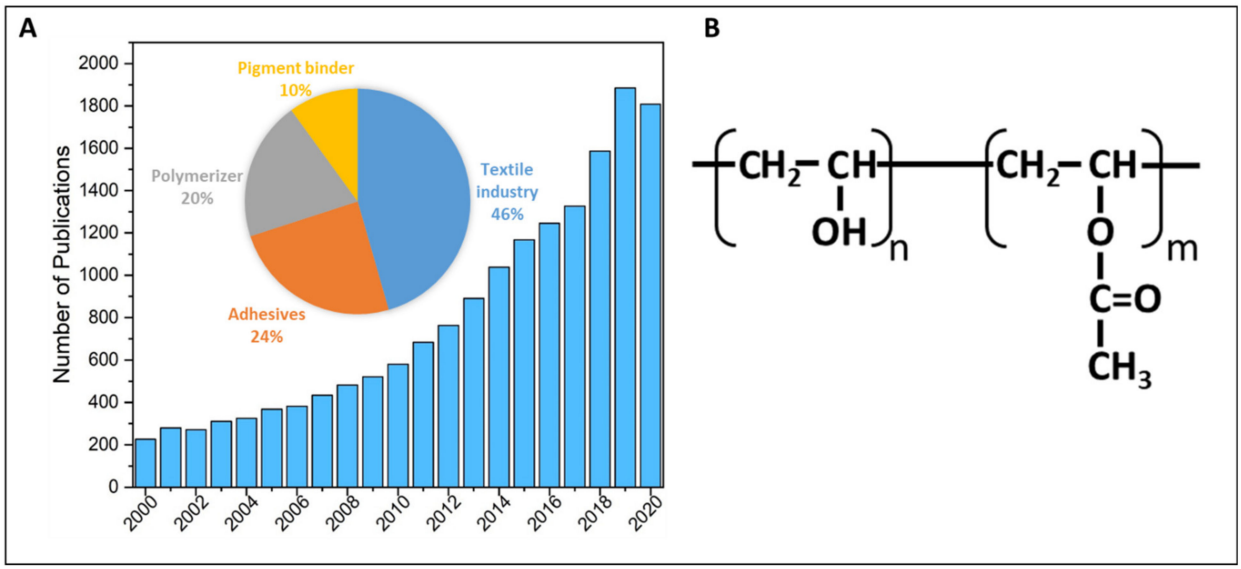

Figure 1. (**A**) Increasing number of publications per year focusing on polyvinyl alcohol (PVA) as well as a pie chart depicting percentage distribution of PVA applications [3] and (**B**) the chemical structure for partially hydrolyzed polyvinyl alcohol-acetate.

Up to 650,000 tons of PVA is produced yearly across the globe [4] and this has been expected to increase 4.09% annually from 2018 to 2023 [5]. In 2018, due to its general increase in usage, PVA was considered to be one of the most ubiquitous pollutants in wastewater [4,6,7]. A thorough understanding of its path to and breakdown within the environment is presently lacking. Although water-soluble, its constituents, such as ethylene (a petroleum-based product), can remain intact within the solvent. Studies have shown ethylene to have negative effects on surrounding organisms, such as plants, which naturally produce and utilize ethylene [8]. Similar to table salt and sugar, PVA dissolves in water, and if the water volume is low, a viscous solution will be formed. The high water volume in WWTPs means the texture of the water should remain unchanged. When PVA is discharged into water bodies, it has the ability to foam due to its surface properties [9]. This can inhibit oxygen transfer, causing irreparable harm to aquatic life [10]. Additionally, because of its hydrophilicity, PVA has the potential to adsorb dangerous chemicals or contaminants [11], such as antibiotics [12] or heavy metals [13–15], at high concentrations. These can then concentrate up food chains [16], posing a threat to the environment, similar to behavior of traditional polluted plastics. WWTPs are known to contain a variety of dangerous contaminants, creating a higher-risk situation for PVA particles passing through [17].

The PVA used in LDPs is composed of PVA polymeric chains with a fraction of polymeric acetate groups (see Figure 1B). This is referred to as partially hydrolyzed PVA, and the percentage of hydrolysis and molecular weights vary with its application [18,19]. The PVA used in LDP blends is typically 88% hydrolyzed [18], while its molecular weight can vary within several ranges, including but not limited to 1000–1,000,000, 10,000–300,000, and 20,000–150,000 Da [19]. Upon contact with water, the presence of polymeric acetate groups enables the water molecules to penetrate the bonds of the outer coating and break it into smaller chains. Once flushed down the drain, partially hydrolyzed PVA chains (See Figure 1B) enter wastewater channels, eventually interacting with WWTPs. The fate of PVA in wastewater treatment systems has been partially explored, with some studies highlighting specific aspects of the wastewater treatment process, but very few studies aimed to establish a complete degradation estimate in conventional WWTP processes from beginning to end.

Available studies suggest that the degradation of PVA occurs under a specific set of circumstances, which may not be ubiquitous within WWTPs or the natural environment. Ultimately, PVA degradation is reported to be a slow process, which greatly depends upon

the surrounding conditions mentioned in Table S2 [20]. Bacteria utilize enzymes to degrade PVA to its constituent form by attacking specific bonds within the polymeric chain [7,21]. Bacteria oxidizes the tertiary carbon atoms, leading to the endo-cleavage of PVA molecules, one of the main degradation routes, which leads to the creation of byproducts such as hydrolyzable hydroxy ketone and 1,3-diketone [20,22]. Other microorganisms mainly utilize PVA as a carbon source; such is the case with the bacteria *Pseudomonas* [20], which generate hydrogen peroxide and other byproducts, including a lower molecular weight PVA [23,24]. Many of these processes can take place simultaneously to begin the degradation of the polymer [20]. While several bacterial species have been documented degrading PVA, these are infrequently found within conventional WWTPs or the environment [20], as are the other optimal circumstances necessary for PVA to completely degrade (Table S1). WWTPs are predominantly designed to remove suspended solids, harmful bacteria, and pollutants of emerging concern [25], but the removal of PVA is still in question due to a lack of comprehensive research. Independent studies have been performed demonstrating removal of PVA using different bacteria, enzymes, and chemical processes [20], but a complete fate assessment in a conventional WWTP or the environment is currently lacking.

In this study, we present a detailed qualitative, quantitative, and spatial analysis on annual mass emissions of PVA in the US, consisting of three defined segments: (i) an online survey involving 500+ respondents investigating their laundry and dish detergent purchasing habits (ii) a United States Geological Survey (USGS) report on water usage, sources, and population; (iii) and a detailed literature review on the degradation of PVA in wastewater, utilizing the data to conduct a subsequent mass balance analysis.

2. Materials and Methods

2.1. Online Survey

An online survey was conducted with 527 respondents, 80% female and 20% male, ages 24–55 years. The survey was directed at the primary decision-maker responsible for purchasing cleaning products in the household. In total, 60% of the responses were from the top 20 designated market areas (DMAs). Survey questions mainly investigated the frequency of buying laundry and dish detergent and the type of detergent bought (single dose pods or detergent bottle) (see Supplemental Information).

2.2. Water Use and Wastewater Generation in the US

State wise data on water use and application for the United States was published in the USGS report titled "Estimated Use of Water in the United States in 2015" [26]. Treatment plants in the US receive wastewater from public facilities, domestic sources, and industrial effluents. Hence, the total water used that ultimately resulted in the generation of wastewater was the sum of public, domestic, and industrial supply (Mgal/d) as shown in Equation (1) which assisted in populating Figure 2.

$$W_{Use,2015} = W_P + W_D + W_I \quad (1)$$

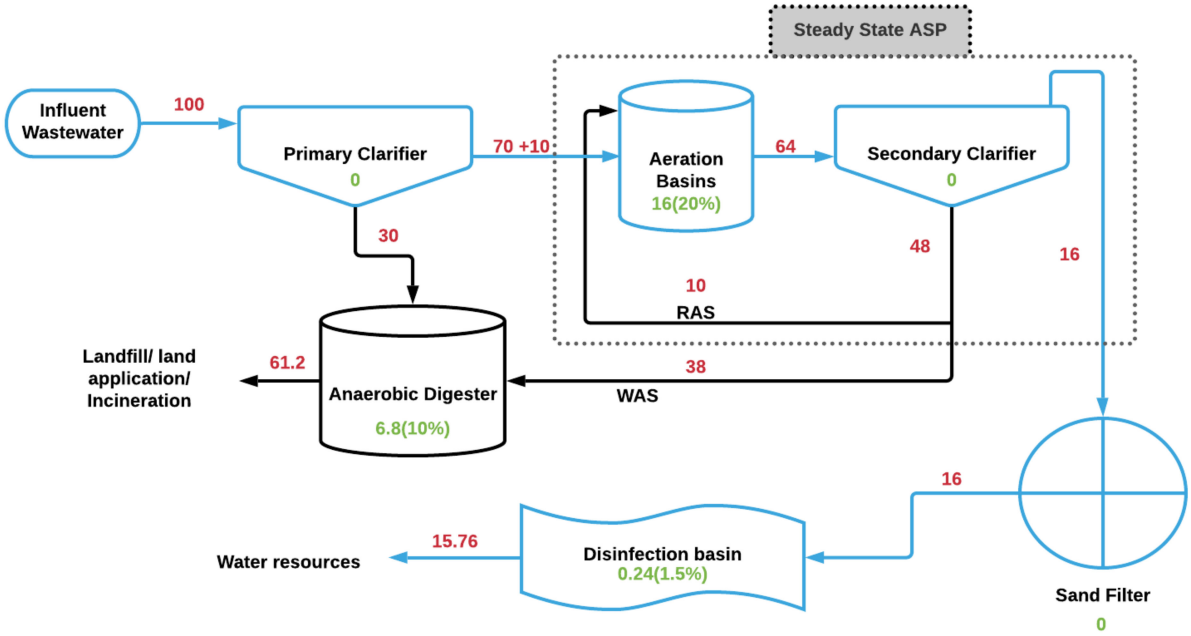

Figure 2. Mass balance of PVA in a conventional activated sludge treatment plant, considering clarifier efficiencies and biodegradation efficiencies. Numbers in red indicate the percentage of PVA in respective treatment streams, and numbers in green represent the amount (% absolute) of degraded PVA in respective sections. RAS and WAS represent return activated sludge and waste activated sludge, respectively. Numbers in parentheses represent the degradation efficiencies of respective sections.

In this equation, W_P, W_D, and W_I stand for public, domestic, and industrial water supplies, respectively.

Not all water used is received by WWTPs as wastewater; there are an estimated 20%–25% in losses [27]. which include but are not limited to leakages of sewers and sanitary sewage outflows.

The total wastewater generated was calculated using Equation (2), assuming a 20% loss in volume. In this case, $WW_{G,2015}$ stands for the wastewater generated for each state.

$$WW_{G,2015} = 0.8 * (W_P + W_D + W_I) \quad (2)$$

Roughly 16,000 US WWTPs treat 34 billion gallons (*BGD*) of wastewater daily [28]. This represents an average of approximately 2.12 billion gallons treated daily per facility. Wastewater treated for each state can be calculated using Equation (3), where N_F and $WW_{T,2015}$ represent the number of wastewater treatment facilities in each state and amount of wastewater treated, respectively, assuming a complete use of operational treatment capacity.

$$WW_{T,2015} = N_F * 2.12\ BGD \quad (3)$$

The untreated wastewater was calculated as the difference between generated and treated wastewater, as shown in Equation (4). Untreated wastewater could be a result of several possibilities, including but not limited to the lack of connectivity between households and WWTPs, improper disposal of wastewater generated, and/or generated wastewater exceeding the operational capacity of a WWTP (current assumption).

$$WW_{UT,2015} = WW_{G,2015} - WW_{T,2015} \quad (4)$$

Subsequently, the percentage of wastewater untreated and treated were calculated as shown in Equations (5) and (6) respectively.

$$WW_{UT,\%} = \frac{WW_{UT,2015}}{WW_{G,2015}} * 100 \quad (5)$$

$$WW_{T,\%} = \frac{WW_{T,2015}}{WW_{G,2015}} * 100 \quad (6)$$

2.3. LDP Usage and Treatment

Laundry pods from three brands and dish pods from two brands were drained, air-dried overnight, and weighed on an Ohaus Adventurer weighing scale (AR1530, China). Laundry pods from different brands (n = 9) and dish pods from varying brands (n = 6) were weighed in triplicate. The average weights for laundry ($M_{L,Avg}$) and dish ($M_{D,Avg}$) pods were 1.0 ± 0.6 g and 0.5 ± 0.2 g, respectively.

Data from the online survey revealed consumption of ~15 billion laundry pods ($N_{L,Avg}$) and ~12 billion dish pods ($N_{D,Avg}$) per year by 126 million households in the US. Using 2015 state-wise population numbers from the USGS report [29] (P_{2015}), the number of per capita pods consumed in the US ($N_{PC,Avg}$) was calculated using Equation (7).

$$N_{PC,Avg} = \frac{N_{L,Avg} + N_{D,Avg}}{P_{2015}} \quad (7)$$

The outer dissolvable coating is composed of PVA and other additives in varying proportions. Based on patents and reports, the PVA ratio (by weight) lies between 65% and 99% of the total outer coating weight [30]. An average mass of PVA in laundry and dish pods was calculated using Equation (8), where f_L and f_D are the fractions of PVA in laundry and dish pods, respectively, in grams.

$$M_{PVA,Avg} = M_{L,Avg} * f_L + M_{D,Avg} * f_D \quad (8)$$

The number of pods (laundry and dish) used by each state was calculated as per Equation (9).

$$N_{Pod} = N_{PC,Avg} * P_{2015} \quad (9)$$

The mass of discarded PVA from laundry pods ($M_{L,G}$) and dish pods ($M_{D,G}$), in each state was expressed as per Equation (10).

$$M_{PVA,G} = N_{Pod} * M_{PVA,Avg} \quad (10)$$

Untreated and treated masses of PVA were calculated as per Equations (11) and (12), respectively.

$$M_{UT,PVA} = M_{PVA,G} * WW_{UT,\ \%} \quad (11)$$

$$M_{T,PVA} = M_{PVA,G} * WW_{T,\ \%} \quad (12)$$

Applying total degradation percentages (solid + aqueous phase) from the modeled scenario to the PVA treated ($M_{T,PVA}$) would result in total PVA emissions from WWTPs.

2.4. GIS and Mapping

Figure 3 was created in Arc GIS pro 2.0.0. Data specific to the US states and environmental emissions of PVA (metric tons/yr) were collected from outside sources. These were then imported into the GIS software program ArcMap.

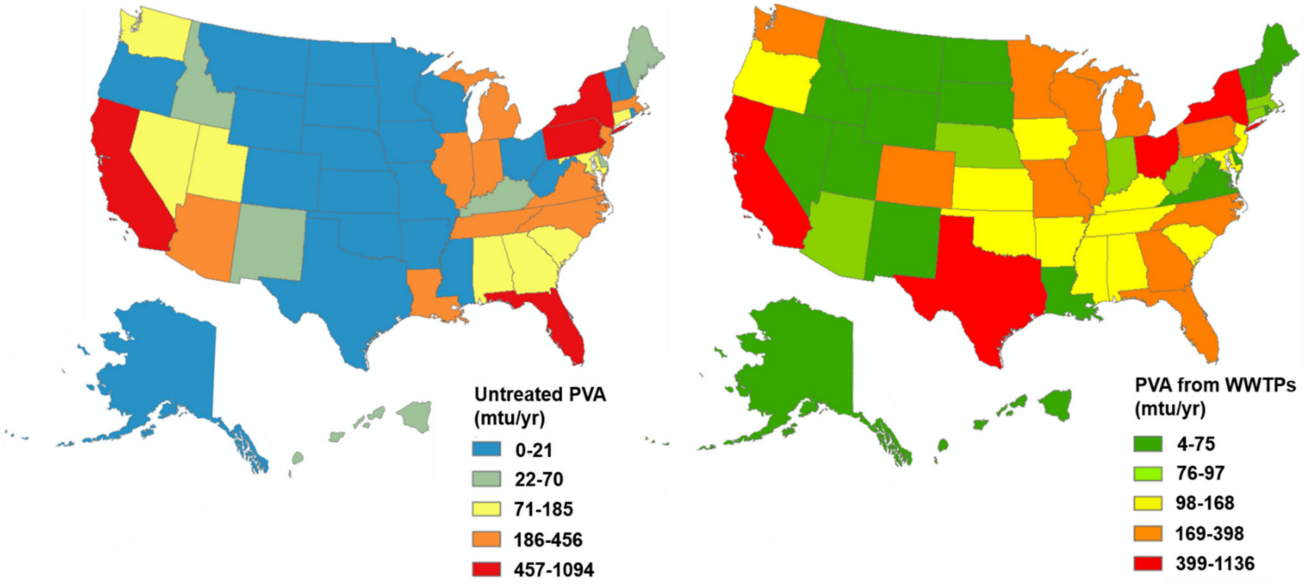

Figure 3. PVA emissions across the U.S. in mtu/yr. The left panel is the spatial distribution of untreated PVA via wastewater that does not reach the treatment plants. The right panel represents the PVA from WWTP effluent streams, including aqueous and sludge disposal routes.

2.5. Literature Review

A comprehensive literature review was performed on the presence and degradation of PVA in US wastewater according to PRISMA guidelines. A detailed breakdown of the publication selection is presented in SI Figure S1. Databases accessed included Google Scholar and Scopus, utilizing the search terms polyvinyl alcohol*; PVA*; Polyvinyl alcohol with results further specified using the constraining term (AND): Pollution*; Degradation*; Biodegradation*; Wastewater*; WWTP*; Sludge*; Sewage*; Effluent*; Influent*; Activated sludge*; US. Papers published between 1950 and 2020 were sought out. These studies were specifically sought out as they aimed to assess the breakdown of PVA within a conventional WWTP by microbial attack or other forces, such as UV radiation or chlorine oxidation. From each relevant paper, the degradation mechanism was extracted as well as the percent degradation, WWTP section (if applicable), the microorganism species, and whether or not the species was adapted to the wastewater itself. As many of the papers included several independent studies relating to PVA degradation in wastewater, a range of percentages were then included to encapsulate all reported values within a single manuscript. Papers with incomplete text or a lack of relevance to wastewater were excluded (see Figure S1).

3. Removal of PVA in WWTPs

The majority of the PVA, generally in the form of greywater from domestic, public, and industrial sources, reaches WWTPs, where it encounters primary treatment, secondary treatment, sludge treatment, and disinfection before leaving the WWTP.

3.1. Primary Treatment

Primary treatment consists of initial screening, grit removal, and a primary clarifier, with the objective of removing coarse solids and other large items [31]. PVA has not been studied in the context of its partitioning and removal in primary treatment within a conventional WWTP. Degrading PVA is a challenge and can contribute to the total chemical oxygen demand (COD) [32] from the incoming wastewater. The most influential mechanism of removal in a primary clarifier is the sorption to the suspended solid particles [33]. However, PVA is a hydrophilic polymer and has a greater affinity toward water, tending to stay in the liquid phase as opposed to solid [34]. It is possible that PVA could form gelatinous consistencies with fats and lipids from influent wastewater, which may increase its partition

toward solid matter and its removal from the aqueous phase. An empirical study analyzing hydrophilic pharmaceuticals and endocrine-disrupting chemicals in wastewater revealed the low sorption capacity of hydrophilic contaminants to solids, requiring relatively longer retention times (5–11 h) for their efficient removal from the aqueous phase [35]. Current literature evidence is not sufficient to establish a definitive path PVA may take in a primary treatment system, but the COD removal efficiency of the primary clarifiers likely eliminates PVA from the aqueous phase [36].

3.2. Secondary Treatment

Secondary treatment is designed to further remove organics and suspended solids [31]. Two main components of secondary treatment are activated sludge processing and the use of the secondary clarifier.

3.2.1. Activated Sludge Process (ASP)

The activated sludge process involves the recycling of the bacteria/microbes present in the sludge, which are sent back into the aeration chambers. These microbes are responsible for the degradation of organic waste and chemicals from the influent wastewater. The bulk of PVA degradation is anticipated to occur at this stage. There are several key factors influencing the biodegradation of PVA in an ASP. One important element is the food-to-microorganisms ratio (F:M), defined as the load of substrate applied daily per unit of biomass [37]. A general F:M ratio lies anywhere between 0.25 and 0.45 [38]. However, an F:M ratio better suited for PVA degradation lies between 0.1 and 0.15 [39]; thus, a higher number of microorganisms is required to fully degrade PVA, compared with conventional domestic sludge. Typical microorganisms may not be able to adequately break down PVA, as the presence of PVA-adapted microbes is necessary for thorough biodegradation of PVA in the ASP [22,39,40]. PVA adaption often requires a lag time spanning over several weeks [22,39], and the ASP can only be PVA-adapted in WWTPs receiving a heavy influx from textile industries, allowing sufficient time for the microbes present at the facility to adapt to the incoming COD. After the lag phase is complete, biodegradation thereafter occurs exponentially [39].

The number of PVA-degrading microbes is limited, and their presence is specific to certain environments and environmental conditions. Schonberger et al. discovered that WWTPs consistent with PVA adaption within the ASP achieved 80% degradation in a 7-day period. Alternatively, the unadapted ASP was consistent with only 18% biodegradation within the same time frame [39]. Hoffman et al. observed similar findings when four different blends of PVA were tested in adapted and unadapted sludge. In unadapted sludge, a degradation of 88 $\pm$ 9% of incoming PVA was achieved in 187 $\pm$ 25 h for four blends, and ~20% was degraded in 25 h [22]. For adapted sludge, 90 $\pm$ 5% was degraded in just 29 $\pm$ 2 h [22]. The above findings utilized 88% hydrolyzed PVA, the same composition as that used in detergent pod coatings [30,41].

The average hydraulic retention time (HRT) in the ASP is approximately 18–24 h, and the sludge retention time (SRT) is 12–15 days [42]. Due to the hydrophilic nature of PVA, the majority of PVA is expected to be in the water phase, in which the HRT would subsequently play a larger role in its degradation.

3.2.2. Secondary Clarifier

The decomposed sludge mixture then enters the secondary clarifier, where the solid waste is given time to settle, allowing the liquid to enter the tertiary treatment stage. In the secondary clarifier, three removal mechanisms may occur, which include volatilization, biodegradation, and adsorption to solids [33]. The current literature does not present evidence of volatilization, biodegradation, or adsorption of PVA in secondary clarifiers. However, since the majority of secondary sludge contains fluid, and the density of PVA is ~1.2 g/cm^3, a fraction of PVA may settle and exit via secondary sludge.

3.3. Tertiary Treatment

Tertiary treatment typically consists of a disinfection chamber and a filtration unit. Older WWTPs use chlorination as an effective disinfectant, along with trickling or gravity filters, due to their moderate efficiency and low cost [43]. These technologies are evolving, as most modern WWTPs rely on advanced oxidation processes (AOPs) and membrane filtration, according to the United States Environmental Protection Agency (USEPA) [44].

3.4. Disinfection

In 2017, Ye et al. studied the comparative effects of using UV, chlorine, and a UV-chlorine combination in PVA degradation/oxidation [45]. Their experiments revealed that 20 min of UV irradiation (Is = 2.6 mW/cm^2) had a minimal effect on the initial concentration of PVA (50 mg/L). Chlorine had a similar result, with just 1.5% of original PVA degraded in 20 min. Chlorine conditions of 20 mg Cl_2/L and a pH of 7 are synchronous with WWTP disinfection conditions in the US [46]. However, a combination of UV-chlorine treatment resulted in 92% degradation within 10 min and 100% in 20 min. Advanced oxidation technologies have been documented to eliminate PVA completely from wastewater in a matter of minutes [15]. These processes can be expensive and thus not cost-effective for the municipality.

In 2004, the US EPA reported that chlorination is the most utilized method for wastewater disinfection (EPA primer, 2014). Given the degradation conditions under chlorine alone, only a minimal amount of PVA coming from domestic wastewaters can be expected to be degraded.

3.5. Filtration

Sand filtration is one of the most widely used filtration techniques due to its low cost of operation and maintenance [47]. Research on the behavior of PVA in a sand or trickling filter system is lacking. However, in cases of missing data, its fate can be predicted based on the behavior of other commonly used hydrophilic materials, such as pharmaceuticals with an octanol-water partitioning coefficient (log Kow) below 2 (see Table 1). A low log Kow indicates the compound's affinity toward water and categorizes the compound as hydrophilic in nature. The table below demonstrates the very low removal efficiencies of widely known hydrophilic pharmaceuticals in a sand filtration system. It should be noted that sand filtration had minimal to no effect on the concentrations of the compounds studied, with a mean and standard deviation of $-0.08\% \pm 20\%$. It is likely that sand filtration would not have a significant effect on PVA concentrations.

Table 1. Removal efficiencies of hydrophilic pharmaceuticals and their log Kow factors in filtration systems.

Compound	RE (%)	log Kow
Ibuprofen	21 [48]	2.48
Gemfibrozil	17 [48]	4.77
Diclofenac	9 [48]	1
Fenofibric acid	5 [48]	1.9
Clofibric acid	15 [48]	2.88
Carbamazepine	1.4 [49]	1.51
Doxycycline	−13 [49]	−0.62
Oxytetracycline	−33 [49]	−0.9
Sulfadiazine	7.7 [49]	−0.09
Acetaminophen	−40 [49]	0.46

3.6. Anaerobic Digestors

Wastewater activated sludge coming from secondary clarifier effluent is treated in an anaerobic digester to reduce the overall volume, destroy pathogens, and control odors [50]. Using anaerobic microbes, the organic matter is further broken down, releasing methane

and water as byproducts. This treated sludge, termed "biosolids", is then safe to be used for agricultural purposes, as an example. In the US, anaerobic digesters are typically catered towards mesophilic (30–37 °C) and thermophilic (50–60 °C) bacteria with retention times of 12–25 and 10–12 days, respectively [50].

Matsumura et al. explored PVA degradability using a lab-scale anaerobic digester with activated sludge obtained from a local WWTP in Japan [51]. Two drastically different molecular weights (14,000 and 2000 Da) were selected for the study. In the first 25 days, both PVA blends showed similar biodegradation results of 12.5%. As time progressed, PVA-14,000 degraded at a much higher rate compared with PVA-2000. After 150 days, PVA-14,000 reached 50% degradation, while PVA-2000 was only 37% degraded [51]. Such low biodegradation rates could be attributed to the usage of PVA-unadapted sludge.

Different PVA-starch blends were studied by Russo et al. under anaerobic conditions. They studied starch to PVA ratios of 90:10, 75:25, 50:50, and 0:100. Anaerobic conditions of 38 ± 5 °C with microbes sourced from activated sludge processes were maintained under nitrogen for up to 100 h. Because PVA used in detergent pods does not contain any starch additives [30], the results of the starch to PVA blend of 0:100 most closely mimicked detergent-derived PVA behavior. The solubilization study yielded lower results for the 0:100 blend, with only 10% of the PVA able to solubilize under anaerobic conditions in 100 h. Its 90:10 counterpart was solubilized up to 60% in the same time allotment [52]. Anaerobic digestion efficacy is often assessed based on the amount of methane and carbon dioxide produced as a byproduct. Upon isolating methane and CO_2 production analysis, the 0:100 blend produced less than 5 mL/g COD, whereas the 90:10 blend produced ~40 mL/g COD [52]. These results highlight the inefficiency of traditional anaerobic digestion methods for the biodegradation of PVA.

Another study analyzing the breakdown of PVA-glycerol-starch blends in anaerobic digestors was conducted by Pšeja et al. in 2006 [53]. After 30 days of incubating PVA blends at 35 ± 2 °C with anaerobic bacterial cultures, the percentage of biodegradation was assessed based on the balance of carbon, biogas, as well as the liquid phase [53]. The bacterial inoculum was sourced from unadapted municipal sludge of a local WWTP. A total of 13 blends were studied, including 85%/15% PVA-glycerol, 70%/15% PVA-starch, and a 75%/15%/10% PVA/starch/glycerol blend. The biodegradation percentages varied from 4.1% to 19.8%. The 85%/15% PVA-glycerol blend degraded the least, at 4.1% in 30 days, whereas the 75%/15%/10% PVA/starch/glycerol blend degraded the most, at 19.8% [53]. According to the authors, high degradation rates (high carbon differential) can be attributed to the biodegradation of starch, not the PVA itself. Since detergent pods do not contain starch additives, high biodegradation rates may not be observed during anaerobic digestion.

4. Estimated Mass Balance

The mass balance presented here is a combination of degradation percentages adopted from Sections 3.1–3.6, as well as a WWTP scenario assumed by Garrido et al. in 2013 [54]. Additionally, WWTP assumptions similar to those of Garrido et al. regarding primary and secondary clarifier COD removal efficiencies were also adopted. A variety of scenarios can be suggested based on a high number of variations in treatments across the US. However, many US WWTPs are older in age and rely on conventional treatment technologies. A conventional activated sludge facility (completely mixed) with primary treatment (screening, grit removal, and primary clarifier), secondary treatment (aeration basins, secondary clarifier, and activated sludge process), tertiary treatment (disinfection and sand filtration), and anaerobic digester was assumed for this study. The COD removal efficiency of the primary clarifier was fixed at 30% and 75% for the secondary clarifier [54]. PVA was considered to be a part of the total COD in this model, and the efficiencies signifying the percentage of the partitioning of COD within the clarifiers were directly applied to PVA. Table 2 indicates the treatment section and the corresponding degradation percentages for the SRT, HRT, and other process conditions based on the literature review within this study. PVA in the

influent wastewater entering primary treatment is considered to be 100%. As PVA passes the primary clarifier, 30% is expected to partition into the solid phase and eventually reach the anaerobic digester. The remaining PVA (70%) in the aqueous phase then enters the activated sludge system (see Table 2).

Table 2. The treatment section, corresponding degradation percentages, SRT, HRT, and other process conditions in a conventional sewage treatment plant. Other processes that do not contribute to degradation are excluded from this table.

Sr. No.	Process	HRT (h)	SRT (days)	Other Conditions	Degradation (%)
1	Activated sludge process	18–24 [42]	12–15 [42]	F:M ratio: 0.25–0.45 [38] PVA unadapted sludge	20
2	Anaerobic digestion	NA	25 [50]	37 °C [50] PVA unadapted sludge	10
3	Disinfection	0.5 [46]	NA	chlorination 20 mgCl_2/L	1.5

NA: Not applicable.

The microbial activity in the aeration basins degrades 20% of the PVA in the presence of unadapted sludge during 18–24 h of HRT. Residual PVA (64%) enters the secondary clarifier, where 75% (48% of total) is partitioned into the sludge phase, and 25% (16% of total) is carried over via the liquid phase to the sand filters. The 48% in the sludge phase is further divided into 21% (10% of total) as return activated sludge (RAS) and 79% (38% of total) as waste activated sludge (WAS). Therefore, 10% of the total PVA is assumed to be in the form of return activated sludge (RAS). RAS values are adopted from the EPA report, in which RAS flow varied between 15% and 127% of the secondary influent flow, with the number eventually settling in the lower 20s [55]. RAS and primary effluent amount to 80% of the total PVA entering the aeration basin.

Solid phase effluent from the primary clarifier (30%) and WAS (38%) are treated in an anaerobic digester, where 10% is degraded (6.8% of the total) and 61.2% of the total is left untreated and ready to be landfilled, land applied, or incinerated.

The aqueous phase, containing 16% of the total PVA reaching the sand filtration stage, enters unaltered into the disinfection basin, where 1.5% is degraded (0.24% of the total) and 15.76% remains intact within the aqueous phase. A detailed mass balance can be found in Figure 2. References for degradation percentages and respective retention times are listed in Table 2.

5. Nationwide PVA Emissions via WWTPs (Effluent + Biosolids)

Referencing the above model, it is estimated that ~61.2% of PVA is emitted via sludge, and ~15.7% is emitted through effluent, with ~77% of the PVA still intact after passing through conventional wastewater treatment (see Figure 2). Once PVA has passed through conventional water treatment, either untreated or within sludge, its journey and fate become important to understand. By accessing data relevant to sewage treatment in US states, we were able to project the amount of intact PVA emitted by each US state. Our research indicates that the Midwest of the US has the lowest amount of untreated PVA, possibly due to lower populations and fewer population-dense areas (see Figure 3, left panel). The Southern and some Western areas of the US have lower to moderate volumes of PVA emissions. However, states such as California, Florida, New York, and Pennsylvania have the highest loadings via untreated wastewater. This may be due to the presence of more metropolitan cities and overworked public facilities as populations grow, causing urban expansion [56].

PVA emissions via WWTP effluent demonstrated slightly different data, with certain regions remaining the same (see Figure 3, right panel). Similar to untreated PVA emissions, much of the Midwest had lower emissions, with the South showing slightly higher numbers, and larger states with high treatment capacities, such as Texas, California, New York, and Florida, having the highest loadings via effluent. It is worth noting that most of

the states with the highest PVA emissions, either untreated or via effluent, have coasts bordering the Pacific or Atlantic Oceans, suggesting a quicker release of PVA into aquatic or marine ecosystems. Previous research has detected wastewater-derived contaminants in the ocean [57], and similar methods could be used to trace the presence of PVA within surrounding marine or terrestrial ecosystems. As human populations and their LDP usage continue to increase, it is expected that wastewater-derived contaminants will also increase [57].

Our data suggest that, on average, only ~10,500 ± 3000 mtu/yr (See Figure 4) of PVA enters treatment infrastructure, and only a fraction of this is biodegraded due to the specificity of conditions required to facilitate complete degradation. Based on the assumed WWTP scenario, 15.76% remains in the aqueous phase (~1600 ± 500 mtu/yr) and 61.2% (6500 ± 1900 mtu/yr) remains in the biosolids exiting the anaerobic digester. Thus, a total of 8100 ± 2400 mtu/yr of PVA is estimated to remain untreated by WWTPs annually in the United States. Of that, 6500 ± 1900 mtu/yr of PVA remains untreated due to lack of treatment capacity or inaccessibility to a functioning WWTP in certain remote communities.

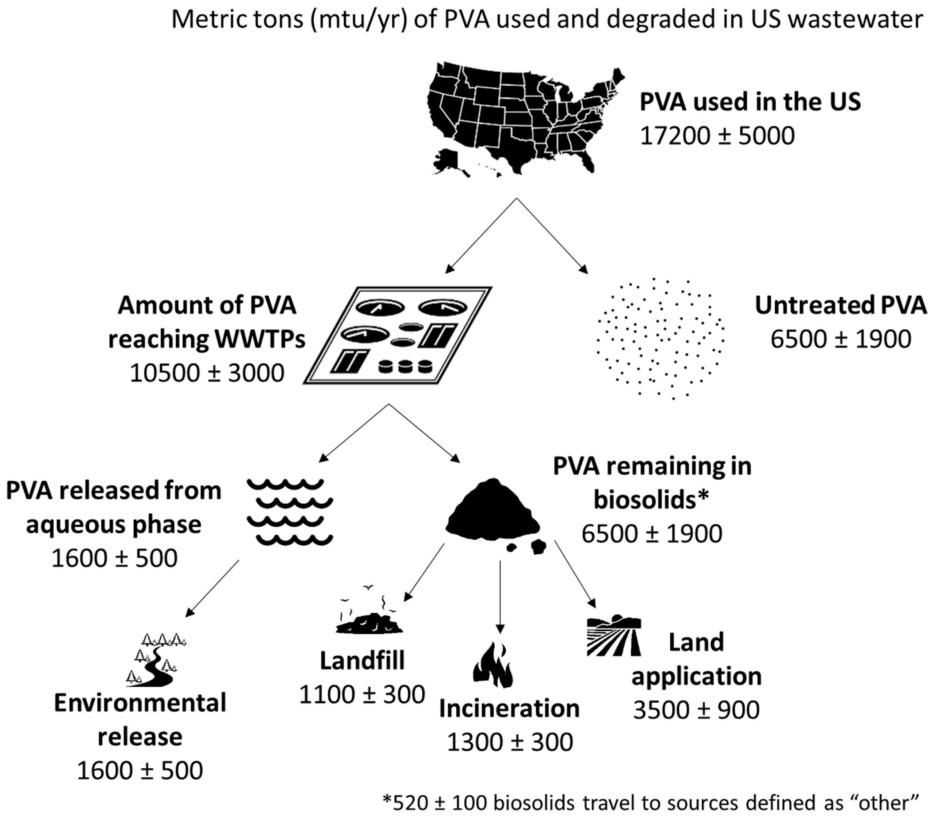

Figure 4. Modeled PVA usage and emissions in metric tons per year (mtu/yr) in the US.

Once biosolids leave a WWTP facility, 50–60% are applied to agricultural lands, 20% are sent to be incinerated, and 17% are sent to a landfill [58]. Each of these locations carries environmental risks associated with the distribution of plastics from biosolids. Initial research chronicling the negative impact of environmental PVA and future areas of study are listed in the following section.

6. Implications

The pathways of sludge have been well documented, as has the ability of WWTPs to act as sources of contaminants and microplastics entering the environment. These emissions can cause deleterious impacts on surrounding ecosystems and the biota within them. PVA that passes through conventional water treatment can similarly pose a threat to the

environment in several ways, once released into the environment or if land applied Our data suggest that around 3500 mtu/yr of PVA is sequestered within agricultural soils in the US. As mentioned previously, ethylene is a byproduct of PVA degradation and is also a hormone utilized by plants. It is unknown if ethylene derived from PVA could affect agricultural yields, but it warrants investigation. The ability for plastic particles to adsorb dangerous contaminants at high concentrations has been documented, but this research is currently lacking as it pertains to PVA. Initial studies revealed that PVA can alter gas exchanges, such as carbon dioxide exchange, affecting aquatic ecosystems [20]. It is also capable of leaching into the groundwater, and it has even been documented to mobilize heavy metals from sediments to water resources [59–61]. Hydrophilic compounds, such as biocides, insecticides, herbicides, flame retardants, corrosion inhibitors, personal care products, and pharmaceuticals are present in wastewater and stormwater [62]. Some of these are proven carcinogens [63] with great aqueous phase stability. As the sorption of organic and inorganic pollutants is not limited to hydrophobic compounds but can also occur with hydrophilic compounds, PVA could act as a vector for transport up the food chain, similarly to more conventional plastics. During such phenomena, the contaminant concentrates, increasing its level of toxicity [61]. This area requires additional research in order to further elucidate the impact of intact PVA on the natural environment.

Prior research has demonstrated that WWTPs are sources of microplastic pollution in natural and built environments. This is due to the fact that microplastics in treated sludge, termed "biosolids", can have a variety of harmful effects on ecosystems beyond contaminant adsorption. A sizable fraction of biosolids is deposited on agricultural soils as they serve as a rich form of fertilizer, ultimately improving soil properties [64]. If biosolids are contaminated with microplastics, the particles destabilize the benefits of sludge by negatively affecting microbial activity, bulk density, and water holding capacity of the soils [57]. A portion of biosolids is also sent to landfills around the US. It is thought that this process sequesters materials in the long term, but landfill leachate has been found to carry microplastics, prompting the consideration of landfills as a source of microplastics into the environment [65]. Lastly, a portion of US biosolids is incinerated. Research has shown that incineration does not terminate plastic waste completely. On the contrary, residual ash can be considered a potential source of microplastic release into the atmosphere or environment [66]. If the plastics are completely incinerated, this process can produce airborne contaminants or pollutants [66]. As demonstrated, incomplete PVA breakdown within conventional water treatment results in a fraction of the material being sequestered within biosolids. The effects and behavior of residual PVA particles within biosolids are not well understood. More research is required to determine their impact on the environment relative to other, more conventional, plastics, whose physical presence in biosolids and ability to adsorb dangerous contaminants creates a threat to ecosystems.

In summary, this research aimed to isolate trends within the current industrial output of PVA used for laundry or dish detergent pods in US wastewater; investigate the components; assess the biodegradability, solubility, and bacterial effect on its structure; and, lastly, outline the potential risks PVA poses as an environmental pollutant. We observed that PVA has low degradation rates within WWTPs; thus, its hydrophilicity and massive production numbers make it a cause for concern as a pollutant in the natural environment. Very little research exists that aims to monitor the biodegradability of PVA in the natural environment. This presents a challenge in determining its role or impact as a pollutant. Research into truly eco-friendly substitutes for PVA is warranted and should be further explored. Improving upon this research is essential for better understanding the link between PVA usage, and public and environmental health.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijerph18116027/s1, Literature review, survey questions, discussion of PVA degrading bacteria, Figure S1: Flow chart flow diagram, Table S1: Bacteria types and percent PVA degradation, Table S2: Soil PVA degradation rates, Table S3: Hydraulic retention times and solid retention times for WWTP segments, PVA Toxicity analysis.

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References

1. Wilcox, C.; Van Sebille, E.; Hardesty, B.D. Threat of plastic pollution to seabirds is global, pervasive, and increasing. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 11899–11904. [CrossRef] [PubMed]
2. Calabrò, P.S.; Grosso, M. Bioplastics and waste management. *Waste Manag.* **2018**, *78*, 800–801. [CrossRef] [PubMed]
3. DeMerlis, C.; Schoneker, D. Review of the oral toxicity of polyvinyl alcohol (PVA). *Food Chem. Toxicol.* **2003**, *41*, 319–326. [CrossRef]
4. Xu, S.; Malik, M.A.; Qi, Z.; Huang, B.; Li, Q.; Sarkar, M. Influence of the PVA fibers and SiO2 NPs on the structural properties of fly ash based sustainable geopolymer. *Constr. Build. Mater.* **2018**, *164*, 238–245. [CrossRef]
5. GlobeNewsWire. *Polyvinyl Alcohol (PVA) Market Size Worth $1.76 Bn by 2023 Witnessing a CAGR of 4.09% during 2018 to 2023—Market Research Future*; GlobeNewsWire: Los Angeles, CA, USA, 2018.
6. Tokiwa, Y.; Kawabata, G.; Jarerat, A. A modified method for isolating poly (vinyl alcohol)-degrading bacteria. *Biotechnol. Lett.* **2001**, *23*, 1937–1941. [CrossRef]
7. Yamatsu, A.; Matsumi, R.; Atomi, H.; Imanaka, T. Isolation and characterization of a novel poly(vinyl alcohol)-degrading bacterium, *Sphingopyxis* sp. PVA3. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 804–811. [CrossRef] [PubMed]
8. Druege, U. Ethylene and plant responses to abiotic stress. *Ethyl. Action Plants* **2006**, 81–118. [CrossRef]
9. Shan, J.; Guan, Y.; Zheng, Q.; Han, J.; Liu, Q.; Pu, Z. Application of urea/H2O2activation-oxidation system in degradation of PVA and desizing of polyester/cotton fabric. *J. Appl. Polym. Sci.* **2009**, *113*, 860–867. [CrossRef]
10. Sun, W.; Tian, J.; Chen, L.; He, S.; Wang, J. Improvement of biodegradability of PVA-containing wastewater by ionizing radiation pretreatment. *Environ. Sci. Pollut. Res.* **2012**, *19*, 3178–3184. [CrossRef]
11. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [CrossRef]
12. Li, J.; Zhang, K.; Zhang, H. Adsorption of antibiotics on microplastics. *Environ. Pollut.* **2018**, *237*, 460–467. [CrossRef]
13. Brennecke, D.; Duarte, B.; Paiva, F.; Caçador, I.; Canning-Clode, J. Microplastics as vector for heavy metal contamination from the marine environment. *Estuar. Coast. Shelf Sci.* **2016**, *178*, 189–195. [CrossRef]
14. Lei, L.; Hu, X.; Yue, P.L.; Bossmann, S.H.; Göb, S.; Braun, A.M. Oxidative degradation of poly vinyl alcohol by the photochemically enhanced Fenton reaction. *J. Photochem. Photobiol. A Chem.* **1998**, *116*, 159–166. [CrossRef]
15. Sun, W.; Chen, L.; Wang, J. Degradation of PVA (polyvinyl alcohol) in wastewater by advanced oxidation processes. *J. Adv. Oxid. Technol.* **2017**, *20*. [CrossRef]
16. Hollman, P.C.H.; Bouwmeester, H.; Peters, R.J.B. *Microplastics in Aquatic Food Chain: Sources, Measurement, Occurrence and Potential Health Risks*; RIKILT-Institute of Food Safety: Wageningen, The Netherlands, 2013; No. 2013.003.
17. Nimonkar, Y.S.; Yadav, B.; Talreja, P.; Sharma, A.; Patil, S.; Saware, S.S.; Ranade, D.R.; Prakash, O. Assessment of the role of wastewater treatment plant in spread of antibiotic resistance and bacterial pathogens. *Indian J. Microbiol.* **2019**, *59*, 261–265. [CrossRef]
18. Chemicals, S.S. SelvolTM Polyvinyl Alcohol Product Information. Available online: https://www.sekisui-sc.com/products/polyvinyl-alcohol/ (accessed on 12 February 2021).
19. United States Patent Application. Process of Making a Water Soluble Pouch. Available online: https://patents.google.com/patent/US20160340068A1/en (accessed on 2 June 2021).
20. Chiellini, E.; Corti, A.; D'Antone, S.; Solaro, R. Biodegradation of poly (vinyl alcohol) based materials. *Prog. Polym. Sci.* **2003**, *28*, 963–1014. [CrossRef]
21. Julinová, M.; Vaňharová, L.; Jurča, M. Water-soluble polymeric xenobiotics—Polyvinyl alcohol and polyvinylpyrrolidone—And potential solutions to environmental issues: A brief review. *J. Environ. Manag.* **2018**, *228*, 213–222. [CrossRef] [PubMed]

22. Hoffmann, J.; Řezníčková, I.; Kozáková, J.; Růžička, J.; Alexy, P.; Bakoš, D.; Precnerová, L. Assessing biodegradability of plastics based on poly (vinyl alcohol) and protein wastes. *Polym. Degrad. Stab.* **2003**, *79*, 511–519. [CrossRef]
23. Watanabe, Y.; Morita, M.; Hamada, N.; Tsujisaka, Y. Formation of hydrogen peroxide by a polyvinyl alcohol degrading enzyme. *Agric. Biol. Chem.* **1975**, *39*, 2447–2448. [CrossRef]
24. Kawai, F.; Hu, X. Biochemistry of microbial polyvinyl alcohol degradation. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 227–237. [CrossRef]
25. Halden, R.U.; Paull, D.H. Co-Occurrence of triclocarban and triclosan in U.S. Water Resources. *Environ. Sci. Technol.* **2005**, *39*, 1420–1426. [CrossRef] [PubMed]
26. Dieter, C.A.; Maupin, M.A.; Caldwell, R.R.; Harris, M.A.; Ivahnenko, T.I.; Lovelace, J.K.; Barber, N.L.; Linsey, K.S. Estimated use of water in the United States in 2015. In *U.S. Geological Survey Circular 1441*; U.S. Geological Survey: Reston, VA, USA, 2017; ISBN 9781411342330.
27. Kaur, R.; Wani, S.P.; Singh, A.K.; Lal, K. Wastewater production, treatment and use in India. In Proceedings of the 2nd Regional Workshop on Safe Use of Wastewater in Agriculture, New Delhi, India, 16–18 May 2012; pp. 1–13.
28. US Environmental Protection Agency. *The Sources and Solutions: Wastewater*; US Environmental Protection Agency: Washington, DC, USA, 2019.
29. Nigel Patrick, S.R. Water Soluble Pouches. Canada Patent CA2463025C, 21 October 2008.
30. Sonune, A.; Ghate, R. Developments in wastewater treatment methods. *Desalination* **2004**, *167*, 55–63. [CrossRef]
31. Magdum, S.S.; Minde, G.P.; Kalyanraman, V. Rapid determination of indirect cod and polyvinyl alcohol from textile desizing wastewater. *Pollut. Res.* **2013**, *32*, 515–519.
32. Katsoyiannis, A.; Zouboulis, A.; Samara, C. Persistent organic pollutants (POPs) in the conventional activated sludge treatment process: Model predictions against experimental values. *Chemosphere* **2006**, *65*, 1634–1641. [CrossRef] [PubMed]
33. Farid, O.; Mansour, F.; Habib, M.; Robinson, J.; Tarleton, S. Investigating the sorption influence of poly(vinyl alcohol) (PVA) at different crosslinking content. *J. Environ. Chem. Eng.* **2016**, *4*, 293–298. [CrossRef]
34. Brandt, E.M.; de Queiroz, F.B.; Afonso, R.J.; Aquino, S.; Chernicharo, C.A. Behaviour of pharmaceuticals and endocrine disrupting chemicals in simplified sewage treatment systems. *J. Environ. Manag.* **2013**, *128*, 718–726. [CrossRef] [PubMed]
35. Sun, M.; Lu, S. Polyvinyl alcohol treatment of textile wastewater under eco-environmental protection. *Ekoloji* **2019**, *28*, 2131–2140.
36. Hamza, R.A.; Sheng, Z.; Iorhemen, O.T.; Zaghloul, M.S.; Tay, J.H. Impact of food-to-microorganisms ratio on the stability of aerobic granular sludge treating high-strength organic wastewater. *Water Res.* **2018**, *147*, 287–298. [CrossRef]
37. State of Michigan Department of Environmental Quality. *Activated Sludge Process Control Training Manual for Wastewater*; Michigan Department of Environmental Quality: Lansing, MI, USA. Available online: https://www.michigan.gov/documents/deq/wrd-ot-activated-sludge-manual_460007_7.pdf (accessed on 2 June 2021).
38. Schonberger, H.; Baumann, A.; Keller, W. Study of microbial degradation of polyvinyl alcohol (PVA) in wastewater treatment plants. *Am. Dyest. Report.* **1997**, *86*, 9–18.
39. Kumar, K.; Singh, G.K.; Dastidar, M.; Sreekrishnan, T. Effect of mixed liquor volatile suspended solids (MLVSS) and hydraulic retention time (HRT) on the performance of activated sludge process during the biotreatment of real textile wastewater. *Water Resour. Ind.* **2014**, *5*, 1–8. [CrossRef]
40. Sekisui Speciality Chemicals. *SelvolTM Polyvinyl Alcohol Product Information*; Sekisui Speciality Chemicals: Houston, TX, USA, 2016.
41. Soriano, G.A.; Erb, M.; Garel, C.; Audic, J.M.; Aguilera, S.G. A Comparative pilot-scale study of the performance of conventional activated sludge and membrane bioreactors under limiting operating conditions. *Water Environ. Res.* **2003**, *75*, 225–231. [CrossRef]
42. USEPA. *Wastewater Technology Fact Sheet Chlorine Disinfection*; USEPA: Washington, DC, USA, 1999.
43. USEPA. *Emerging Technologies for Wastewater Treatment and In-Plant Wet Weather Management*; USEPA: Washington, DC, USA, 2012; pp. 1–144.
44. Ye, B.; Li, Y.; Chen, Z.; Wu, Q.-Y.; Wang, W.-L.; Wang, T.; Hu, H.-Y. Degradation of polyvinyl alcohol (PVA) by UV/chlorine oxidation: Radical roles, influencing factors, and degradation pathway. *Water Res.* **2017**, *124*, 381–387. [CrossRef] [PubMed]
45. Kelkar, V.P.; Rolsky, C.B.; Pant, A.; Green, M.D.; Tongay, S.; Halden, R.U. Chemical and physical changes of microplastics during sterilization by chlorination. *Water Res.* **2019**, *163*, 114871. [CrossRef] [PubMed]
46. Four Effective Processes to Treat Wastewater. Available online: https://eponline.com/articles/2018/02/08/four-effective-processes-to-treat-wastewater.aspx (accessed on 3 January 2021).
47. Drewes, J.E. Chapter 4.1 Removal of pharmaceutical residues during wastewater treatment. In *Analysis, Removal, Effects and Risk of Pharmaceuticals in the Water Cycle—Occurrence and Transformation in the Environment*; Elsevier: Amsterdam, The Netherlands, 2007; pp. 427–449. [CrossRef]
48. Gao, P.; Ding, Y.; Li, H.; Xagoraraki, I. Occurrence of pharmaceuticals in a municipal wastewater treatment plant: Mass balance and removal processes. *Chemosphere* **2012**, *88*, 17–24. [CrossRef]
49. Costa, A.; Ely, C.; Pennington, M.; Rock, S.; Staniec, C.; Turgeon, J. *Anaerobic Digestion and Its Applications*; USEPA: Washington, DC, USA, 2015; p. 15.
50. Matsumura, S.; Kurita, H.; Shimokobe, H. Anaerobic biodegradability of polyvinyl alcohol. *Biotechnol. Lett.* **1993**, *15*, 749–754. [CrossRef]

51. Russo, M.A.; O'Sullivan, C.; Rounsefell, B.; Halley, P.J.; Truss, R.; Clarke, W. The anaerobic degradability of thermoplastic starch: Polyvinyl alcohol blends: Potential biodegradable food packaging materials. *Bioresour. Technol.* **2009**, *100*, 1705–1710. [CrossRef]
52. Pšeja, J.; Charvátová, H.; Hruzík, P.; Hrncirik, J.; Kupec, J. Anaerobic biodegradation of blends based on polyvinyl alcohol. *J. Polym. Environ.* **2006**, *14*, 185–190. [CrossRef]
53. Garrido, J.M.; Fdz-Polanco, F. Working with energy and mass balances: A conceptual framework to understand the limits of municipal wastewater treatment. *Water Sci. Technol.* **2013**, *67*, 2294–2301. [CrossRef]
54. West, A.W. *Operational Control of The Return Activated Sludge*; USEPA: Washington, DC, USA, 1973.
55. Deslauriers, S.A.; Kanzaki, M.; Bulkley, J.W.; Keoleian, G.A. *US Wastewater Treatment*; Center for Sustainable System: Ann Arbor, MI, USA, 2020.
56. Rolsky, C.; Kelkar, V.; Driver, E.; Halden, R.U. Municipal sewage sludge as a source of microplastics in the environment. *Curr. Opin. Environ. Sci. Health* **2020**, *14*, 16–22. [CrossRef]
57. Venkatesan, A.K.; Halden, R.U. Brominated flame retardants in U.S. biosolids from the EPA national sewage sludge survey and chemical persistence in outdoor soil mesocosms. *Water Res.* **2014**, *55*, 133–142. [CrossRef]
58. Hamad, D.; Mehrvar, M.; Dhib, R. Experimental study of polyvinyl alcohol degradation in aqueous solution by UV/H_2O_2 process. *Polym. Degrad. Stab.* **2014**, *103*, 75–82. [CrossRef]
59. Chowdhury, N.K.; Ismail, A.F.; Beg, M.D.H.; Hegde, G.; Gohari, R.J. Polyvinyl alcohol/polysaccharide hydrogel graft materials for arsenic and heavy metal removal. *New J. Chem.* **2015**, *39*, 5823–5832. [CrossRef]
60. Liu, G.; Zhu, Z.; Yang, Y.; Sun, Y.; Yu, F.; Ma, J. Sorption behavior and mechanism of hydrophilic organic chemicals to virgin and aged microplastics in freshwater and seawater. *Environ. Pollut.* **2019**, *246*, 26–33. [CrossRef]
61. Spahr, S.; Teixidó, M.; Sedlak, D.L.; Luthy, R.G. Hydrophilic trace organic contaminants in urban stormwater: Occurrence, toxicological relevance, and the need to enhance green stormwater infrastructure. *Environ. Sci. Water Res. Technol.* **2019**, *6*, 15–44. [CrossRef]
62. Wielsøe, M.; Long, M.; Ghisari, M.; Bonefeld-Jørgensen, E.C. Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers in vitro. *Chemosphere* **2015**, *129*, 239–245. [CrossRef]
63. Alvarez-Campos, O.; Evanylo, G.K. Biosolids improve urban soil properties and vegetable production in urban agriculture. *Urban Agric. Reg. Food Syst.* **2019**, *4*, 1–11. [CrossRef]
64. He, P.; Chen, L.; Shao, L.; Zhang, H.; Lü, F. Municipal solid waste (MSW) landfill: A source of microplastics? Evidence of microplastics in landfill leachate. *Water Res.* **2019**, *159*, 38–45. [CrossRef]
65. Yang, Z.; Lü, F.; Zhang, H.; Wang, W.; Shao, L.; Ye, J.; He, P. Is incineration the terminator of plastics and microplastics? *J. Hazard. Mater.* **2021**, *401*, 123429. [CrossRef]
66. Gallo, F.; Fossi, C.; Weber, R.; Santillo, D.; Sousa, J.; Ingram, I.; Nadal, A.; Romano, D. Marine litter plastics and microplastics and their toxic chemicals components: The need for urgent preventive measures. *Environ. Sci. Eur.* **2018**, *30*, 1–14. [CrossRef]

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Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk

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Abstract: The widespread use of plastics determines the inevitable human exposure to its by-products, including microplastics (MPs), which enter the human organism mainly by ingestion, inhalation, and dermal contact. Once internalised, MPs may pass across cell membranes and translocate to different body sites, triggering specific cellular mechanisms. Hence, the potential health impairment caused by the internalisation and accumulation of MPs is of prime concern, as confirmed by numerous studies reporting evident toxic effects in various animal models, marine organisms, and human cell lines. In this pilot single-centre observational prospective study, human breastmilk samples collected from N. 34 women were analysed by Raman Microspectroscopy, and, for the first time, MP contamination was found in 26 out of 34 samples. The detected microparticles were classified according to their shape, colour, dimensions, and chemical composition. The most abundant MPs were composed of polyethylene, polyvinyl chloride, and polypropylene, with sizes ranging from 2 to 12 µm. MP data were statistically analysed in relation to specific patients' data (age, use of personal care products containing plastic compounds, and consumption of fish/shellfish, beverages, and food in plastic packaging), but no significant relationship was found, suggesting that the ubiquitous MP presence makes human exposure inevitable.

Keywords: microplastics; human breastmilk; Raman microspectroscopy; infants' nutrition

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1. Introduction

The global production of plastics has reached the impressive amount of more than 350 million tons per year. This is the result of the massive demand for this material, which has been considered, until now, the golden choice in terms of durability, usability, and versatility for a huge variety of applications and consumer products [1–3]. This widespread use of plastics also led to their accumulation in landfills and in the natural environment. In fact, as a consequence of the extensive production and employment of single-use products, which represent >40% of manufactured plastics, 250,000 tons of plastic litter is estimated to be floating in the oceans [4]. Although several countries are introducing new regulations on plastic waste management and recycling strategies, it has to be noted that in 2018 in Europe, only 32.5% of post-consumer waste plastic was recycled, while 24.9% was accumulated in landfills [5].

Environmental plastic contamination derives from several factors, including mismanaged plastic waste, fishing nets in the sea, and different household and commercial activities, such as washing synthetic textiles, road markings, tires, marine coatings, personal care products, and plastic pellets [6,7]. In particular, after being released into the environment, plastic products undergo a degradation process caused by the action of atmospheric agents, such as waves, abrasion, UV radiation, and photo-oxidation, in combination with biological processes, which leads to the formation of microplastics (MPs) [8]. MPs range from 5 millimetres to 100 nanometres and are classified as primary or secondary based on their source of release into the environment: primary MPs are purposely manufactured at sizes <5 mm to be employed for commercial purposes (such as glitter in cosmetic products and microbeads in cleansers, scrubs, and dish scrubbing pads), while secondary MPs are generated by the previously described environmental degradation processes of larger plastic items [9,10].

The ubiquitous occurrence of MPs in the environment determines inevitable human exposure, mainly by three routes: ingestion, inhalation, and dermal contact. Among all of them, ingestion is considered the major route, with an estimated intake of 39 ÷ 52 thousand MPs per person per year [11–13]. Once internalised, MPs may pass across cell membranes [14], followed by accumulation or elimination by the onset of specific cellular mechanisms. All of these processes are mainly related to MPs' size, which cannot exceed 10–15 μm.

The potential health impairment caused by the internalisation and accumulation of MPs is of prime concern. Although information is still lacking on this topic, several studies reported evident toxic effects in various animal models, marine organisms, and human cell lines [15–17], showing that MPs, once internalised, are not inert as previously supposed and likely trigger local or systemic responses.

Given the strong concern related to the effects of MPs on animal and human health, the use of reliable and objective techniques for MP detection and characterisation is crucial. Among all of the exploited techniques, Raman Microspectroscopy (RMS) can be considered the gold standard, since it lets researchers characterise not only the morphological features of microparticles but also their chemical composition in terms of both polymer matrices and pigments. Moreover, RMS presents the advantage of enabling the analysis of MPs as small as ~2 μm directly on filtration membranes, thanks to the high potential of light scattering [18–20]. Recently, our research group, for the first time, detected the presence of MPs in human placenta samples; this study, carried out by Raman Microspectroscopy, received extensive attention, since the delicate role played by this organ may be perturbed by the presence of MPs [21].

Based on these impressive results, we decided to investigate the contamination of microplastics in breastmilk to assess another MP exposure route in the extremely vulnerable population of infants. For this purpose, in the present study, milk samples collected from 34 consenting patients were analysed by Raman Microspectroscopy, and, for the first time, in most of the analysed samples, the presence of MPs was detected. The relevance of this research lies in breastmilk being the gold standard for infants' nutrition. Moreover, it reflects both the mother's and infant's postnatal exposure, and hence, it represents an optimal matrix for contaminant biomonitoring [22]. In fact, milk consists of protein and fat globules in a carbohydrate-based suspension and represents a favourable environment for the lipophilic nature of MPs and other chemicals. In this regard, it is noteworthy that several studies reported the contamination of breastmilk by phthalates, heavy metals, and perfluorinated compounds [23–25].

2. Materials and Methods

2.1. Cohort Selection

This was a pilot observational descriptive study in a prospective and single-centre cohort. It was approved by the Ethical Committee Lazio 1 (Protocol N. 708/CE Lazio 1; 24 May 2021), and it was carried out in full accordance with ethical principles, including The

Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. A dedicated cohort of N. 34 patients, all characterised by pregnancies without complications, was enrolled at Ospedale Fatebenefratelli Isola Tiberina (Rome, Italy). Exclusion criteria were: (a) medically prescribed special diets within 4 weeks prior to delivery; (b) diarrhoea or severe constipation within 2 weeks prior to delivery; (c) use of antibiotics within 2 weeks prior to delivery; (d) use of drugs affecting intestinal resorption, e.g., activated charcoal or cholestyramine, within 2 weeks prior to delivery; (e) diagnosis of a gastrointestinal pathology, e.g., ulcerative colitis or Crohn's disease, except for appendectomy; (f) cancer, HIV, or any other serious illness demanding medical treatment; (g) invasive or abrasive dental treatments within 2 weeks prior to delivery; (h) alcohol abuse (defined as Alcohol Use Disorder Identification test >10); and (i) current or recent (within the previous 4 weeks) participation in a clinical trial. Patients who decided to participate were asked to sign an informed consent form. Patients were also asked to fill in a questionnaire to record their food consumption, with a special focus on fish, shellfish, and foods employing packaging, and the use of personal care products from 7 days before the expected date of delivery to 7 days after.

2.2. Sample Collection

Breastmilk samples were collected 1 week after delivery at the Department of Obstetrics and Gynaecology of San Giovanni Calibita Fatebenefratelli Hospital (Rome, Italy). Patients were guided on a manual expression procedure, recommended by the World Health Organization and described in a document released by the Italian Ministry of Health [26], which uses manual expression to obtain the maximum milk output and to avoid pain or damage to the breast tissue. No breast pumps were allowed to avoid contamination from its plastic components. Briefly, the manual expression procedure consists of cupping the breast with one hand, with the other forming a C-shape with the thumb and the forefinger, 3–4 cm from the base of the nipple; then, pressure is applied by pushing towards the ribcage, squeezing with the thumb and forefinger, and finally releasing the pressure. The sequence of pressure, squeeze, and release was repeated until obtaining an adequate amount of milk. Milk samples were placed into glass flasks, weighed, and then stored at -20 °C until processing. Each sample contained an average amount of 4.16 ± 1.73 g of breastmilk.

2.3. Sample Digestion and Filtration

In order to remove organic components from milk samples, a digestion protocol was set up and performed at the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental Sciences, Università Politecnica delle Marche (Ancona, Italy). A 10% KOH solution prepared using 1.6 µm filtered deionised water and KOH tablets (Sigma-Aldrich) was added to each flask in a ratio of sample to KOH of 1:10 (w/v). Flasks were sealed and incubated at 40 °C for 48 h [27]. Digestates were then filtered through a 1.6 µm pore-size filter membrane (Whatman GF/A) by a vacuum pump connected to a filter funnel. Filter membranes were dried at room temperature and stored in glass Petri dishes until Raman Microspectroscopy (RMS) analysis.

2.4. Detection and Identification of MPs by Raman Microspectroscopy

RMS analysis was performed by using an XploRA Nano Raman Microspectrometer (Horiba Scientific) at the ARI Laboratory of Università Politecnica delle Marche (Ancona, Italy). All filter membranes, including those deriving from the procedural blanks, were inspected by visible light using a $\times 10$ objective (Olympus MPLAN10$\times$/0.25). The detected MPs were morphologically characterised by a $\times 100$ objective (Olympus MPLAN100$\times$/0.90) and then directly analysed on the filter by RMS (spectral range 200–1800 cm^{-1}, 532 nm or 785 nm laser diode, 600 lines per mm grating). Spectra were dispersed onto a 16-bit dynamic range Peltier-cooled CCD detector; the spectrometer was calibrated to the 520.7 cm^{-1} line of silicon prior to spectral acquisition. To reduce noise and enhance spectrum quality, raw

Raman spectra were subjected to polynomial baseline correction and vector normalisation (Labspec 6 software, Horiba Scientific). The polymer matrix of the detected particles was identified by comparing the collected Raman spectra with spectral libraries of polymers and pigments obtained by measuring standard polymers/compounds (KnowItAll software, John Wiley & Sons, Inc., Hoboken, NJ, USA) [28,29]. Similarities of more than 80 of the Hit Quality Index (HQI) were considered satisfactory.

2.5. Quality Assurance and Control (QA/QC)

Efforts were adopted to avoid microplastic contamination during sample collection, storage, processing, and analysis. To this aim, a plastic-free protocol was adopted during all phases of the experiment, and a dedicated room was used for the digestion of milk samples, filtration, and RMS analysis steps. Routinely employed plastic tools were replaced with sterilised glass ones. Cotton laboratory coats and single-use latex gloves were worn during all phases of the experiment. All liquids, including ethanol for cleaning and deionised water for cleaning and preparation of all solutions, were filtered through 1.6 μm pore-size filter membranes (Whatman GF/A). Work surfaces were thoroughly washed with 70% ethanol prior to starting all procedures and during the experimental time. Glassware and instruments, including scissors and tweezers, were washed using dishwashing liquid, triple rinsed with 70% ethanol, and finally rinsed with 1.6 μm filtered deionised water.

Moreover, environmental and procedural blanks were prepared and thoroughly analysed to detect microplastic contamination deriving from the laboratory environment and from other external sources. As regards environmental blanks, a filter membrane soaked with 1.6 μm filtered deionised water was placed into an uncovered Petri dish and positioned each day in the above-mentioned dedicated room. A procedural blank was also prepared together with every batch of samples following the exact same procedure as samples, but without adding milk. The filters deriving from environmental and procedural blanks were first inspected by stereomicroscope.

2.6. Statistical Analysis

Data analysis was performed by using the statistical software package Prism6 (Graphpad Software, Inc., San Diego, CA, USA). Normality was checked by the D'Agostino and Pearson omnibus normality test. Chi-square test, Student's *t*-test, and one-way analysis of variance (ANOVA) were performed to compare data accordingly. The significance threshold was set at $p < 0.05$.

3. Results

In the present study, N. 34 breastmilk samples were investigated by RMS for the presence of microplastics. MP contamination was found in 26 out of 34 women. As regards QA/QC protocols, the analysis of environmental (N. 14) and procedural (N. 9) blanks was performed. In the environmental blanks, only fibres, for a total of N. 16, ranging from 571 μm to 3000 μm, were found. Conversely, no MP contamination was found in the filters from the procedural blanks. Given the dimensions of the fibres, which are not compatible with the translocation into breastmilk, and the absence of fibres in the analysed milk samples, there was no need to blank-correct the results.

All details about the identified microparticles (such as morphology, dimensions, colour, polymer matrix, and pigment) are listed in Table 1.

Table 1. Information about patients (age, quantity of milk sample, and abundance of MPs) and morphological and chemical features of the identified microparticles.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#1	28	8.44	2	0.24	irregular fragment	~10 µm	orange	nitrocellulose	
					irregular fragment	~6 µm	orange	polyethylene	
#2	32	4.92	1	0.20	irregular fragment	~3 µm	blue	polyethylene	
#3	32	5.83	1	0.17	irregular fragment	~6 µm	black	polyvinyl chloride	
#4	38	5.09	2	0.39	irregular fragment	~2 µm	red	polyvinyl chloride	
					irregular fragment	~3 µm	blue	polypropylene	
#5	36	7.08	2	0.28	irregular fragment	~6 µm	red	chlorinated polyethylene	
					sphere	~5 µm	grey	polypropylene	
#6	40	1.95	4	2.05	irregular fragment	~5 µm	light blue	polyvinyl chloride	
					irregular fragment	~1 µm	blue		Pigment Blue 29 (C.I. Constitution 77007)
					irregular fragment	~10 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
					irregular fragment	~10 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
#7	38	3.49	5	1.43	sphere	~5 µm	brown	polyvinyl alcohol	
					irregular fragment	~3 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
					irregular fragment	~10 µm	brown/grey	nitrocellulose	
					sphere	~2 µm	blue		Pigment Blue 29 (C.I. Constitution 77007)
					irregular fragment	~10 µm	light blue	polypropylene	

Table 1. *Cont.*

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#8	36	4.21	2	0.47	irregular fragment	~2 μm	red		Pigment Red 101/102 (C.I. Constitution 77491)
					irregular fragment	~10 μm	red		Pigment Red 101/102 (C.I. Constitution 77491)
#9	45	2.81	3	1.07	irregular fragment	~4 μm	red		Pigment Red 101/102 (C.I. Constitution 77491)
					irregular fragment	~5 μm	yellow/orange	polyethylene	
					irregular fragment	~3 μm	light blue	polyvinyl chloride	
#10	34	5.58	2	0.36	irregular fragment	~6 μm	orange	polyethylene	
					irregular fragment	~6 μm	blue	polypropylene	
#11	39	2.32	0	0					
#12	32	3.55	2	0.56	irregular fragment	~2 μm	black	polyethylene	
					irregular fragment	~10 μm	green	poly(ethylene-co-vinyl acetate)	Pigment Green 7 (C.I. Constitution 74260
#13	41	2.76	1	0.36	irregular fragment	~12 μm	blue	polyethylene	
#14	50	4.50	3	0.67	irregular fragment	~3 μm	brown		Pigment Yellow 43 / Brown 6 (C.I. Constitution 77492)
					irregular fragment	~3 μm	light blue	polyvinyl chloride	
					sphere	~4 μm	grey	polypropylene	
#15	33	5.99	2	0.33	irregular fragment	~5 μm	orange	polyethylene	
					irregular fragment	~6 μm	red	polyvinyl chloride	

Table 1. *Cont.*

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#16	37	4.65	2	0.43	irregular fragment	~10 µm	blue	polyethylene	
					irregular fragment	~2 µm	transparent	polyethylene	
#17	32	1.64	2	1.22	sphere	~5 µm	transparent	polyethylene	
					irregular fragment	~5 µm	brown		Pigment Red 101/102 (C.I. Constitution 77491)
#18	41	3.10	1	0.32	irregular fragment	~8 µm	black	poly(ethyl methacrylate)	
#19	38	6.06	2	0.33	irregular fragment	~8 µm	orange	nitrocellulose	
					irregular fragment	~2 µm	blue/green	polypropylene	
#20	37	4.19	3	0.71	irregular fragment	~3 µm	magenta	polyvinyl chloride	
					irregular fragment	~12 µm	light blue	polyvinyl chloride	
					irregular fragment	~12 µm	green	polyvinyl chloride	
#21	40	2.36	1	0.42	irregular fragment	~5 µm	blue	acrylonitrile butadiene styrene	
#22	41	5.47	0	0					
#23	35	3.82	0	0					
#24	48	2.53	5	1.98	irregular fragment	~2 µm	orange	polystyrene	
					irregular fragment	~10 µm	yellow	polyethylene	
					irregular fragment	~12 µm	transparent	polyethylene	
					irregular fragment	~4 µm	light blue	polypropylene	
					irregular fragment	~5 µm	brown	polyester	

Table 1. *Cont.*

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#25	39	7.51	1	0.13	irregular fragment	~10 µm	blue	polyamide	
#26	37	5.65	2	0.35	irregular fragment	~6 µm	white/transparent	polyethylene	
					irregular fragment	~4 µm	light blue	polypropylene	
#27	31	3.06	1	0.33	irregular fragment	~5 µm	brown	polycarbonate	
#28	35	3.54	0	0					
#29	35	2.68	1	0.37	irregular fragment	~2 µm	light blue	polyethylene	
#30	47	1.84	5	2.72	irregular fragment	~7 µm	white/transparent	polyethylene	
					irregular fragment	~8 µm	yellow/brown	polyethylene	
					irregular fragment	~2 µm	white	polyethylene	
					irregular fragment	~4 µm	orange	high-density polyethylene	
					irregular fragment	~4 µm	blue	polyvinyl chloride	
#31	49	3.85	0	0					
#32	42	4.21	0	0					
#33	45	5.10	0	0					
#34	42	1.54	0	0					

For clarity, in Figure 1, the microphotographs and corresponding Raman spectra of some selected MPs found in the analysed samples are reported.

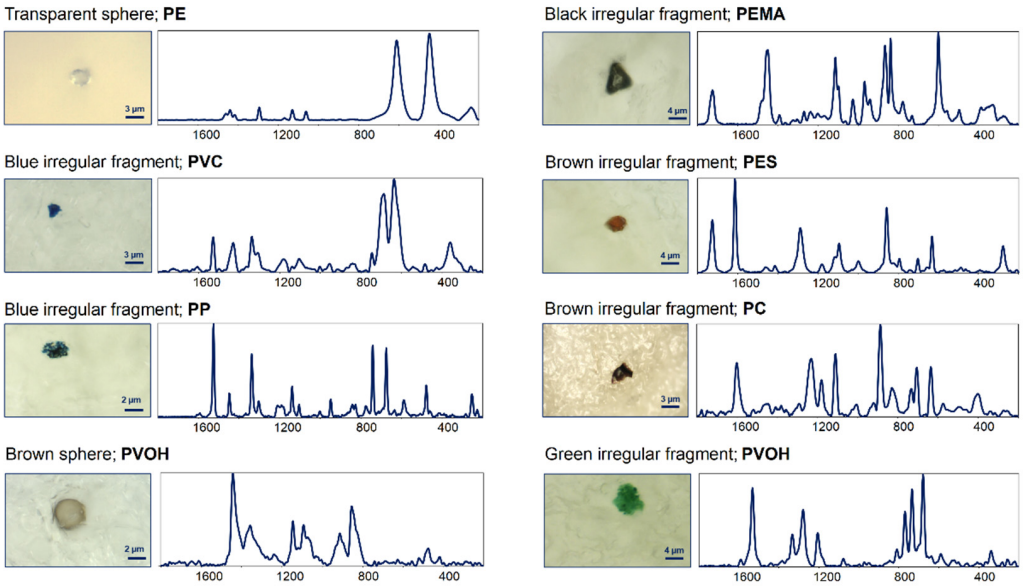

Figure 1. Microphotographs and Raman spectra (wavenumbers, cm^{-1}) of some selected MPs found in the analysed breastmilk samples. PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; PVOH: polyvinyl alcohol; PEVA: poly(ethylene-co-vinyl acetate); PEMA: poly(ethyl methacrylate); PES: polyester, and PC: polycarbonate.

The detected microparticles were classified according to their shape, colour, dimensions, and chemical composition (Figure 2). As regards the shape, only irregular fragments and spheres were found, while no films or fibres were identified (Figure 2A). Moreover, most of the identified MPs were pigmented (ca. 90%), with blue and orange/yellow being the most abundant colours (ca. 36% and ca. 17%, respectively; Figure 2B). As regards MPs' dimensions, almost half of them (ca. 47%) were in the range of 4–9 µm; ca. 29% were ≤3 µm, while ca. 24% were ≥10 µm (Figure 2C). For 48 out of the total 58 identified MPs, the polymer matrix was also identified, while for the remaining ones, the contribution of the pigments used for plastic staining was the only signal in the collected Raman spectra [21,30,31]. Within the identified polymer matrices, the most abundant ones were polyethylene (PE, 38%), polyvinyl chloride (PVC, 21%), and polypropylene (PP, 17%) (Figure 2D).

The statistical relationship between data related to patients and the presence/number of MPs was also investigated. In particular, the following patient-related parameters were analysed: patient's age; use of personal care products containing plastic compounds (including lotions, soaps, and toothpaste); and consumption, in the 7 days prior to the expected date of delivery and 7 days after, of fish/shellfish, beverages in plastic bottles, and food in plastic packaging. In Figure 3A–E, the statistical analysis of the percentages of women with and without MPs in their milk, divided according to each of the above-defined parameters, is reported. As regards 'patient's age', women were divided into three groups as follows: ≤35 years old, 36–40 years old, and ≥41 years old; no statistically significant difference was observed among groups ($p = 0.648$; Figure 3A). Moreover, women's habits of food consumption and use of personal care products were investigated, and in this case as well, no statistically significant difference among groups was observed ('fish/shellfish consumption', $p = 0.961$, Figure 3B; 'personal care product with plastics', $p = 0.1611$, Figure 3C; 'beverages in plastic bottles', $p = 0.9107$, Figure 3D; and 'food in plastic packaging', $p = 0.2963$, Figure 3E). For a deeper analysis, each of the above-defined parameters was also considered in relation to the number of detected MPs (Figure 3F–J); as expected, no significant difference among groups was revealed (one-way ANOVA or Student's *t*-test).

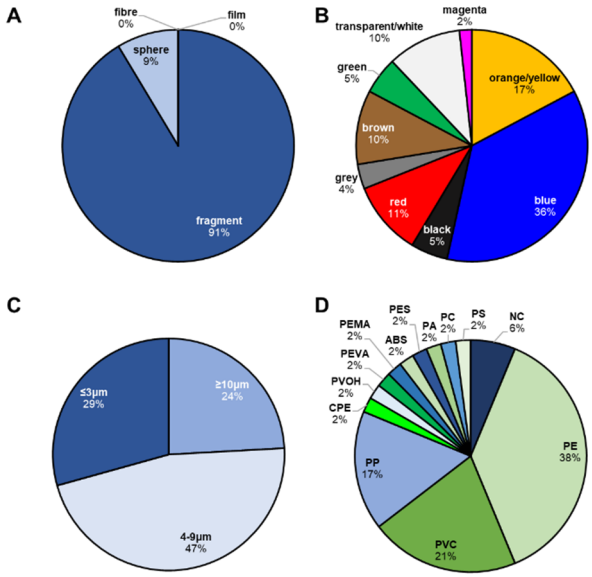

Figure 2. Percentage abundances of identified shapes (**A**), colours (**B**), dimensions (**C**), and polymer matrices (**D**). PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; CPE: chlorinated polyethylene; PVOH: polyvinyl alcohol; PEVA: poly(ethylene-co-vinyl acetate); PEMA: poly(ethyl methacrylate); ABS: acrylonitrile butadiene styrene; PES: polyester; PA: polyamide; PC: polycarbonate; PS: polystyrene, and NC: nitrocellulose.

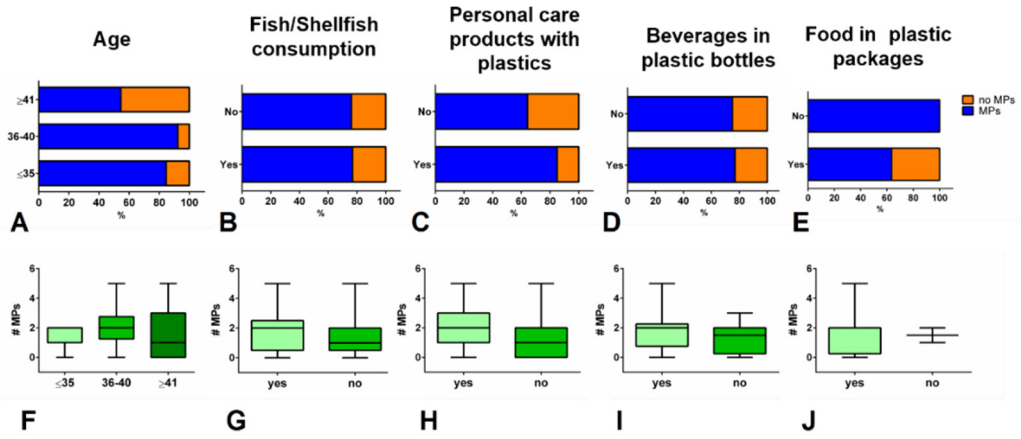

Figure 3. Percentage abundances of samples with (MPs) and without (no MPs) microplastics, divided according to the following selected parameters: (**A**) age of patient; (**B**) consumption of fish/shellfish in the 7 days prior to the expected date of delivery and 7 days after; (**C**) use of personal care products with plastic compounds in the 7 days prior to the expected date of delivery and 7 days after; (**D**) consumption of beverages in plastic bottles in the 7 days prior to the expected date of delivery and 7 days after, and (**E**) consumption of food in plastic packaging in the 7 days prior to the expected date of delivery and 7 days after. Number of identified microplastics divided according to the above-defined parameters (**F**–**J**) (box charts: centre line marks the median, edges indicate the 5th and 95th percentiles, and whiskers indicate the minimum and maximum values).

4. Discussion

Breastmilk represents the best standard nutrition for infants, thanks to its provision of nutrients and enhancement of the immune system [24]. Hence, assessing its quality in terms of possible contamination is mandatory. In fact, mothers are exposed daily to a great variety of chemicals present in the environment, for example, through food, beverages, and personal care products, and for this reason, breastmilk may be contaminated by these compounds, likely impacting children's health [32]. To date, the presence of

polychlorinated bisphenyls (PCBs), organochlorine pesticides, polybrominated diphenyl esters (PBDEs), phthalates and phthalate metabolites, per- and polyfluoroalkyl substances (PFASs), phenols, and metals have been detected in human milk [33]. In fact, since most of these contaminants are lipophilic and have a tendency to deposit in adipose tissue, they may be translocated to milk during lactation [34,35]. It is known that early life stages are the most sensitive to PCBs' toxic effects, which mainly consist of a severe impact on endocrine and cognitive systems, leading to reduced IQ and altered behaviour [36]. Similarly, PBDEs are recognised as neurotoxic, especially in children, with effects on motor, cognitive, and behavioural development [37]. Phthalates have been reported in the literature to negatively impact male reproductive functionality [38]; moreover, childhood exposure to phthalates was shown to increase the risk of allergic diseases and altered physical/neurocognitive development [39,40].

It is noteworthy that most of these environmental pollutants are also able to interact with MPs by several sorption mechanisms, which depend on polymer size, shape, density, colour, and chemical composition [41]; for example, phthalate esters have shown major sorption on polystyrene, polyethylene, and polyvinyl chloride microparticles [42]. Hence, since MPs are ubiquitous environmental contaminants and represent potential vectors for toxic organic compounds with known health impairment effects, their detection in biological matrices is actually of great concern [15–17].

In this study, for the first time, MPs were found in breastmilk samples; it should be stated that the number of microparticles that we detected could be underestimated, since only an aliquot of ~4 g of milk was considered for each sample. MPs were characterised by RMS and classified in terms of shape, dimensions, colour, and chemical composition. In this regard, almost all MPs were blue and orange/yellow irregular fragments with dimensions ranging from 2 μm to 12 μm, consistent with translocation mechanisms. In accordance with other studies reported in the literature, the most abundant polymers were polyethylene, polyvinyl chloride, and polypropylene [43,44].

Several MP routes of exposure have been reported in the literature, including inhalation, dermal contact, and ingestion, with the latter being considered the most impactful, with an estimated total intake of around 39–52 thousand per person per year [12,13]. Once ingested/inhaled, MPs can be internalised in human tissues [11]. At the gastrointestinal level, they may pass through the epithelium by endocytosis mechanisms or by paracellular diffusion, after which they are translocated by dendritic cells through the lymphatic circulation and reach the circulatory system [11]. As regards the respiratory system, inhaled MPs likely penetrate the lower respiratory tract, characterised by a thin mucus layer, and spread into the bloodstream after cellular uptake or paracellular diffusion [45].

Currently, there is growing scientific evidence about MPs in humans. Schwabl et al. reported the detection of MPs in human stool [46], while, as a further measure, Ibrahim et al. described the presence of MPs in human colectomy samples, proving that MPs in part cross the intestinal barrier [47]. As evidence of inhalation exposure, Amato-Lourenço et al. detected, in human lung tissue, <5.5 μm polymeric MPs and fibres ranging from 8.12 to 16.8 μm [48]. We recently found MPs in the human placenta, which represents the interface between the foetus and the mother exposed to the external environment [21], results that were also confirmed by Braun et al. [49]. Very recently, the presence of plastic particles in human blood finally proved the transport of MPs in the bloodstream to every body site [50].

As regards the mammary gland, two hypothetical pathways have been suggested for the translocation of exogenous particles from the bloodstream to breast milk: the mammary epithelial cell-dependent and the immune cell-dependent pathways, with the latter being particularly relevant in the case of inhaled particles [23–25,51,52]. Hence, a possible association between the presence of MPs in breastmilk and specific information regarding mothers' habits (such as the consumption of fish and shellfish, beverages in plastic bottles, and food in plastic packaging and the use of personal care products containing plastic compounds in the 7 days prior to the expected date of delivery and the 7 days after) was

investigated, but no relationship was found between MP presence/number and each of the above-mentioned parameters.

The lack of association with the use of personal care products is likely explained by considering that dermal contact has a minor impact as an exposure route, since only particles <100 nanometres can cross the dermal barrier [11]. Conversely, the absence of a relation with mothers' food habits is more difficult to explain, since the major route of MP exposure is represented by ingestion. In fact, numerous food-related sources of MPs have been reported, including fish, shellfish, and human essential daily consumables, such as table salt, sugar, bottled water, milk, honey, plastic teabags, and to a greater extent, plastic kitchen tools, plates, and packaging [53]. Hence, our findings suggest that, since MPs are ubiquitous in the environment, exposure to these microparticles is inevitable, and, for this reason, it is impossible to isolate a specific source among the complex set of faced exposures.

5. Conclusions

The evidence of MPs in human breastmilk, coupled with the previous discovery of these microparticles in the human placenta, represents a great concern, since it impacts the extremely vulnerable population of infants. In fact, the chemicals possibly contained in foods, beverages, and personal care products consumed by breastfeeding mothers may be transferred to the offspring, potentially exerting a toxic effect. Hence, it is mandatory to increase efforts in scientific research to deepen the knowledge of the potential health impairment caused by MP internalisation and accumulation, especially in infants, and to assess innovative, useful ways to reduce exposure to these contaminants during pregnancy and lactation.

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References

1. Jadhav, E.B.; Sankhla, M.S.; Bhat, R.A.; Bhagat, D.S. Microplastics from food packaging: An overview of human consumption, health threats, and alternative solutions. *Environ. Nanotechnol. Monit. Manag.* **2021**, *16*, 100608. [CrossRef]
2. Lithner, D.; Larsson, Å.; Dave, G. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Sci. Total Environ.* **2011**, *409*, 3309–3324. [CrossRef] [PubMed]
3. *Plastics Europe Plastics—The Facts 2019 An Analysis of European Plastics Production, Demand and Waste Data*; Royal Society of Chemistry: Lodon, UK, 2019.
4. Eriksen, M.; Lebreton, L.C.M.; Carson, H.S.; Thiel, M.; Moore, C.J.; Borerro, J.C.; Galgani, F.; Ryan, P.G.; Reisser, J. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE* **2014**, *9*, e111913. [CrossRef]
5. Conti, I.; Simioni, C.; Varano, G.; Brenna, C.; Costanzi, E.; Neri, L.M. Legislation to limit the environmental plastic and microplastic pollution and their influence on human exposure. *Environ. Pollut.* **2021**, *288*, 117708. [CrossRef]
6. Browne, M.A.; Crump, P.; Niven, S.J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R. Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environ. Sci. Technol.* **2011**, *45*, 9175–9179. [CrossRef] [PubMed]

7. Salvador Cesa, F.; Turra, A.; Baruque-Ramos, J. Synthetic fibers as microplastics in the marine environment: A review from textile perspective with a focus on domestic washings. *Sci. Total Environ.* **2017**, *598*, 1116–1129. [CrossRef] [PubMed]
8. Hanun, J.N.; Hassan, F.; Jiang, J.-J. Occurrence, fate, and sorption behavior of contaminants of emerging concern to microplastics: Influence of the weathering/aging process. *J. Environ. Chem. Eng.* **2021**, *9*, 106290. [CrossRef]
9. Kannan, K.; Vimalkumar, K. A Review of Human Exposure to Microplastics and Insights Into Microplastics as Obesogens. *Front. Endocrinol.* **2021**, *12*, 724989. [CrossRef]
10. Sridharan, S.; Kumar, M.; Singh, L.; Bolan, N.S.; Saha, M. Microplastics as an emerging source of particulate air pollution: A critical review. *J. Hazard. Mater.* **2021**, *418*, 126245. [CrossRef]
11. Prata, J.C.; da Costa, J.P.; Lopes, I.; Duarte, A.C.; Rocha-Santos, T. Environmental exposure to microplastics: An overview on possible human health effects. *Sci. Total Environ.* **2020**, *702*, 134455. [CrossRef]
12. Prata, J.C. Airborne microplastics: Consequences to human health? *Environ. Pollut.* **2018**, *234*, 115–126. [CrossRef] [PubMed]
13. Cox, K.D.; Covernton, G.A.; Davies, H.L.; Dower, J.F.; Juanes, F.; Dudas, S.E. Human Consumption of Microplastics. *Environ. Sci. Technol.* **2019**, *53*, 7068–7074. [CrossRef] [PubMed]
14. Alimba, C.G.; Faggio, C.; Sivanesan, S.; Ogunkanmi, A.L.; Krishnamurthi, K. Micro(nano)-plastics in the environment and risk of carcinogenesis: Insight into possible mechanisms. *J. Hazard. Mater.* **2021**, *416*, 126143. [CrossRef]
15. Danopoulos, E.; Twiddy, M.; West, R.; Rotchell, J.M. A rapid review and meta-regression analyses of the toxicological impacts of microplastic exposure in human cells. *J. Hazard. Mater.* **2021**, 127861. [CrossRef]
16. Han, Y.; Lian, F.; Xiao, Z.; Gu, S.; Cao, X.; Wang, Z.; Xing, B. Potential toxicity of nanoplastics to fish and aquatic invertebrates: Current understanding, mechanistic interpretation, and meta-analysis. *J. Hazard. Mater.* **2022**, *427*, 127870. [CrossRef]
17. Yin, K.; Wang, Y.; Zhao, H.; Wang, D.; Guo, M.; Mu, M.; Liu, Y.; Nie, X.; Li, B.; Li, J.; et al. A comparative review of microplastics and nanoplastics: Toxicity hazards on digestive, reproductive and nervous system. *Sci. Total Environ.* **2021**, *774*, 145758. [CrossRef]
18. Käppler, A.; Fischer, D.; Oberbeckmann, S.; Schernewski, G.; Labrenz, M.; Eichhorn, K.-J.; Voit, B. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Anal. Bioanal. Chem.* **2016**, *408*, 8377–8391. [CrossRef]
19. Ribeiro-Claro, P.; Nolasco, M.M.; Araújo, C. Characterization of Microplastics by Raman Spectroscopy. *Compr. Anal. Chem.* **2017**, *75*, 119–151. [CrossRef]
20. Di Renzo, L.; Mascilongo, G.; Berti, M.; Bogdanović, T.; Listeš, E.; Brkljača, M.; Notarstefano, V.; Gioacchini, G.; Giorgini, E.; Olivieri, V.; et al. Potential Impact of Microplastics and Additives on the Health Status of Loggerhead Turtles (Caretta caretta) Stranded Along the Central Adriatic Coast. *Water Air Soil Pollut.* **2021**, *232*, 98. [CrossRef]
21. Ragusa, A.; Svelato, A.; Santacroce, C.; Catalano, P.; Notarstefano, V.; Carnevali, O.; Papa, F.; Rongioletti, M.C.A.; Baiocco, F.; Draghi, S.; et al. Plasticenta: First evidence of microplastics in human placenta. *Environ. Int.* **2021**, *146*, 106274. [CrossRef]
22. Eidelman, A.I.; Schanler, R.J.; Johnston, M.; Landers, S.; Noble, L.; Szucs, K.; Viehmann, L. Breastfeeding and the Use of Human Milk. *Pediatrics* **2012**, *129*, e827–e841. [CrossRef]
23. Llorca, M.; Farré, M.; Picó, Y.; Teijón, M.L.; Álvarez, J.G.; Barceló, D. Infant exposure of perfluorinated compounds: Levels in breast milk and commercial baby food. *Environ. Int.* **2010**, *36*, 584–592. [CrossRef] [PubMed]
24. LaKind, J.S.; Verner, M.-A.; Rogers, R.D.; Goeden, H.; Naiman, D.Q.; Marchitti, S.A.; Lehmann, G.M.; Hines, E.P.; Fenton, S.E. Current Breast Milk PFAS Levels in the United States and Canada: After All This Time, Why Don't We Know More? *Environ. Health Perspect.* **2022**, *130*, 025002. [CrossRef] [PubMed]
25. Jian, J.M.; Chen, D.; Han, F.J.; Guo, Y.; Zeng, L.; Lu, X.; Wang, F. A short review on human exposure to and tissue distribution of per- and polyfluoroalkyl substances (PFASs). *Sci. Total Environ.* **2018**, *636*, 1058–1069. [CrossRef]
26. Ministero della Salute Allattare al Seno-Un Investimento Per la Vita. 2019. Available online: https://www.salute.gov.it/portale/documentazione/p6_2_5_1.jsp?lingua=italiano&id=303 (accessed on 1 June 2022).
27. Karami, A.; Golieskardi, A.; Choo, C.K.; Romano, N.; Ho, Y.B.; Salamatinia, B. A high-performance protocol for extraction of microplastics in fish. *Sci. Total Environ.* **2017**, *578*, 485–494. [CrossRef]
28. Dong, M.; Zhang, Q.; Xing, X.; Chen, W.; She, Z.; Luo, Z. Raman spectra and surface changes of microplastics weathered under natural environments. *Sci. Total Environ.* **2020**, *739*, 139990. [CrossRef]
29. SLOPP Library of Microplastics. Available online: https://rochmanlab.com/slopp-and-slopp-e-raman-spectral-libraries-for-microplastics-research (accessed on 1 June 2022).
30. Imhof, H.K.; Laforsch, C.; Wiesheu, A.C.; Schmid, J.; Anger, P.M.; Niessner, R.; Ivleva, N.P. Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes. *Water Res.* **2016**, *98*, 64–74. [CrossRef]
31. Stoye, D.; Freitag, W. *Paints, Coatings and Solvents*; Wiley: Hoboken, NJ, USA, 1998; ISBN 9783527288632.
32. Karthikeyan, B.S.; Ravichandran, J.; Aparna, S.R.; Samal, A. ExHuMId: A curated resource and analysis of Exposome of Human Milk across India. *Chemosphere* **2021**, *271*, 129583. [CrossRef]
33. Lehmann, G.M.; LaKind, J.S.; Davis, M.H.; Hines, E.P.; Marchitti, S.A.; Alcala, C.; Lorber, M. Environmental Chemicals in Breast Milk and Formula: Exposure and Risk Assessment Implications. *Environ. Health Perspect.* **2018**, *126*, 096001. [CrossRef]
34. Mead, M.N. Contaminants in Human Milk: Weighing the Risks against the Benefits of Breastfeeding. *Environ. Health Perspect.* **2008**, *116*, A426–A434. [CrossRef]
35. Vasios, G.; Kosmidi, A.; Kalantzi, O.-I.; Tsantili-Kakoulidou, A.; Kavantzas, N.; Theocharis, S.; Giaginis, C. Simple physicochemical properties related with lipophilicity, polarity, molecular size and ionization status exert significant impact on the transfer of drugs and chemicals into human breast milk. *Expert Opin. Drug Metab. Toxicol.* **2016**, *12*, 1273–1278. [CrossRef] [PubMed]

36. Lundqvist, C.; Zuurbier, M.; Leijs, M.; Johansson, C.; Ceccatelli, S.; Saunders, M.; Schoeters, G.; Ten Tusscher, G.; Koppe, J. The effects of PCBs and dioxins on child health. *Acta Paediatr.* **2006**, *95*, 55–64. [CrossRef] [PubMed]
37. Gibson, E.; Siegel, E.; Eniola, F.; Herbstman, J.; Factor-Litvak, P. Effects of Polybrominated Diphenyl Ethers on Child Cognitive, Behavioral, and Motor Development. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1636. [CrossRef] [PubMed]
38. Main, K.M.; Mortensen, G.K.; Kaleva, M.M.; Boisen, K.A.; Damgaard, I.N.; Chellakooty, M.; Schmidt, I.M.; Suomi, A.-M.; Virtanen, H.E.; Petersen, J.H.; et al. Human Breast Milk Contamination with Phthalates and Alterations of Endogenous Reproductive Hormones in Infants Three Months of Age. *Environ. Health Perspect.* **2006**, *114*, 270–276. [CrossRef]
39. Braun, J.M.; Sathyanarayana, S.; Hauser, R. Phthalate exposure and children's health. *Curr. Opin. Pediatr.* **2013**, *25*, 247–254. [CrossRef]
40. Huang, H.-B.; Chen, H.-Y.; Su, P.-H.; Huang, P.-C.; Sun, C.-W.; Wang, C.-J.; Chen, H.-Y.; Hsiung, C.A.; Wang, S.-L. Fetal and Childhood Exposure to Phthalate Diesters and Cognitive Function in Children Up to 12 Years of Age: Taiwanese Maternal and Infant Cohort Study. *PLoS ONE* **2015**, *10*, e0131910. [CrossRef]
41. Rodrigues, J.P.; Duarte, A.C.; Santos-Echeandía, J.; Rocha-Santos, T. Significance of interactions between microplastics and POPs in the marine environment: A critical overview. *TrAC Trends Anal. Chem.* **2019**, *111*, 252–260. [CrossRef]
42. Liu, F.; Liu, G.; Zhu, Z.; Wang, S.; Zhao, F. Interactions between microplastics and phthalate esters as affected by microplastics characteristics and solution chemistry. *Chemosphere* **2019**, *214*, 688–694. [CrossRef]
43. Jones, J.I.; Vdovchenko, A.; Cooling, D.; Murphy, J.F.; Arnold, A.; Pretty, J.L.; Spencer, K.L.; Markus, A.A.; Vethaak, A.D.; Resmini, M. Systematic Analysis of the Relative Abundance of Polymers Occurring as Microplastics in Freshwaters and Estuaries. *Int. J. Environ. Res. Public Health* **2020**, *17*, 9304. [CrossRef]
44. Bajt, O. From plastics to microplastics and organisms. *FEBS Open Bio* **2021**, *11*, 954–966. [CrossRef]
45. Mowat, A.M. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat. Rev. Immunol.* **2003**, *3*, 331–341. [CrossRef] [PubMed]
46. Schwabl, P.; Köppel, S.; Königshofer, P.; Bucsics, T.; Trauner, M.; Reiberger, T.; Liebmann, B. Detection of Various Microplastics in Human Stool. *Ann. Intern. Med.* **2019**, *171*, 453. [CrossRef] [PubMed]
47. Ibrahim, Y.S.; Tuan Anuar, S.; Azmi, A.A.; Wan Mohd Khalik, W.M.A.; Lehata, S.; Hamzah, S.R.; Ismail, D.; Ma, Z.F.; Dzulkarnaen, A.; Zakaria, Z.; et al. Detection of microplastics in human colectomy specimens. *JGH Open* **2021**, *5*, 116–121. [CrossRef] [PubMed]
48. Amato-Lourenço, L.F.; Carvalho-Oliveira, R.; Júnior, G.R.; dos Santos Galvão, L.; Ando, R.A.; Mauad, T. Presence of airborne microplastics in human lung tissue. *J. Hazard. Mater.* **2021**, *416*, 126124. [CrossRef] [PubMed]
49. Braun, T.; Ehrlich, L.; Henrich, W.; Koeppel, S.; Lomako, I.; Schwabl, P.; Liebmann, B. Detection of Microplastic in Human Placenta and Meconium in a Clinical Setting. *Pharmaceutics* **2021**, *13*, 921. [CrossRef]
50. Leslie, H.A.; van Velzen, M.J.M.; Brandsma, S.H.; Vethaak, A.D.; Garcia-Vallejo, J.J.; Lamoree, M.H. Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* **2022**, 107199. [CrossRef]
51. Cai, J.; Zang, X.; Wu, Z.; Liu, J.; Wang, D. Translocation of transition metal oxide nanoparticles to breast milk and offspring: The necessity of bridging mother-offspring-integration toxicological assessments. *Environ. Int.* **2019**, *133*, 105153. [CrossRef]
52. Yang, L.; Kuang, H.; Zhang, W.; Wei, H.; Xu, H. Quantum dots cause acute systemic toxicity in lactating rats and growth restriction of offspring. *Nanoscale* **2018**, *10*, 11564–11577. [CrossRef]
53. Senathirajah, K.; Attwood, S.; Bhagwat, G.; Carbery, M.; Wilson, S.; Palanisami, T. Estimation of the mass of microplastics ingested—A pivotal first step towards human health risk assessment. *J. Hazard. Mater.* **2021**, *404*, 124004. [CrossRef]

polymers

MDPI

Article

Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk

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Abstract: The widespread use of plastics determines the inevitable human exposure to its by-products, including microplastics (MPs), which enter the human organism mainly by ingestion, inhalation, and dermal contact. Once internalised, MPs may pass across cell membranes and translocate to different body sites, triggering specific cellular mechanisms. Hence, the potential health impairment caused by the internalisation and accumulation of MPs is of prime concern, as confirmed by numerous studies reporting evident toxic effects in various animal models, marine organisms, and human cell lines. In this pilot single-centre observational prospective study, human breastmilk samples collected from N. 34 women were analysed by Raman Microspectroscopy, and, for the first time, MP contamination was found in 26 out of 34 samples. The detected microparticles were classified according to their shape, colour, dimensions, and chemical composition. The most abundant MPs were composed of polyethylene, polyvinyl chloride, and polypropylene, with sizes ranging from 2 to 12 µm. MP data were statistically analysed in relation to specific patients' data (age, use of personal care products containing plastic compounds, and consumption of fish/shellfish, beverages, and food in plastic packaging), but no significant relationship was found, suggesting that the ubiquitous MP presence makes human exposure inevitable.

Keywords: microplastics; human breastmilk; Raman microspectroscopy; infants' nutrition

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1. Introduction

The global production of plastics has reached the impressive amount of more than 350 million tons per year. This is the result of the massive demand for this material, which has been considered, until now, the golden choice in terms of durability, usability, and versatility for a huge variety of applications and consumer products [1–3]. This widespread use of plastics also led to their accumulation in landfills and in the natural environment. In fact, as a consequence of the extensive production and employment of single-use products, which represent >40% of manufactured plastics, 250,000 tons of plastic litter is estimated to be floating in the oceans [4]. Although several countries are introducing new regulations on plastic waste management and recycling strategies, it has to be noted that in 2018 in Europe, only 32.5% of post-consumer waste plastic was recycled, while 24.9% was accumulated in landfills [5].

Environmental plastic contamination derives from several factors, including mismanaged plastic waste, fishing nets in the sea, and different household and commercial activities, such as washing synthetic textiles, road markings, tires, marine coatings, personal care products, and plastic pellets [6,7]. In particular, after being released into the environment, plastic products undergo a degradation process caused by the action of atmospheric agents, such as waves, abrasion, UV radiation, and photo-oxidation, in combination with biological processes, which leads to the formation of microplastics (MPs) [8]. MPs range from 5 millimetres to 100 nanometres and are classified as primary or secondary based on their source of release into the environment: primary MPs are purposely manufactured at sizes <5 mm to be employed for commercial purposes (such as glitter in cosmetic products and microbeads in cleansers, scrubs, and dish scrubbing pads), while secondary MPs are generated by the previously described environmental degradation processes of larger plastic items [9,10].

The ubiquitous occurrence of MPs in the environment determines inevitable human exposure, mainly by three routes: ingestion, inhalation, and dermal contact. Among all of them, ingestion is considered the major route, with an estimated intake of 39 ÷ 52 thousand MPs per person per year [11–13]. Once internalised, MPs may pass across cell membranes [14], followed by accumulation or elimination by the onset of specific cellular mechanisms. All of these processes are mainly related to MPs' size, which cannot exceed 10–15 μm.

The potential health impairment caused by the internalisation and accumulation of MPs is of prime concern. Although information is still lacking on this topic, several studies reported evident toxic effects in various animal models, marine organisms, and human cell lines [15–17], showing that MPs, once internalised, are not inert as previously supposed and likely trigger local or systemic responses.

Given the strong concern related to the effects of MPs on animal and human health, the use of reliable and objective techniques for MP detection and characterisation is crucial. Among all of the exploited techniques, Raman Microspectroscopy (RMS) can be considered the gold standard, since it lets researchers characterise not only the morphological features of microparticles but also their chemical composition in terms of both polymer matrices and pigments. Moreover, RMS presents the advantage of enabling the analysis of MPs as small as ~2 μm directly on filtration membranes, thanks to the high potential of light scattering [18–20]. Recently, our research group, for the first time, detected the presence of MPs in human placenta samples; this study, carried out by Raman Microspectroscopy, received extensive attention, since the delicate role played by this organ may be perturbed by the presence of MPs [21].

Based on these impressive results, we decided to investigate the contamination of microplastics in breastmilk to assess another MP exposure route in the extremely vulnerable population of infants. For this purpose, in the present study, milk samples collected from 34 consenting patients were analysed by Raman Microspectroscopy, and, for the first time, in most of the analysed samples, the presence of MPs was detected. The relevance of this research lies in breastmilk being the gold standard for infants' nutrition. Moreover, it reflects both the mother's and infant's postnatal exposure, and hence, it represents an optimal matrix for contaminant biomonitoring [22]. In fact, milk consists of protein and fat globules in a carbohydrate-based suspension and represents a favourable environment for the lipophilic nature of MPs and other chemicals. In this regard, it is noteworthy that several studies reported the contamination of breastmilk by phthalates, heavy metals, and perfluorinated compounds [23–25].

2. Materials and Methods

2.1. Cohort Selection

This was a pilot observational descriptive study in a prospective and single-centre cohort. It was approved by the Ethical Committee Lazio 1 (Protocol N. 708/CE Lazio 1; 24 May 2021), and it was carried out in full accordance with ethical principles, including The

Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. A dedicated cohort of N. 34 patients, all characterised by pregnancies without complications, was enrolled at Ospedale Fatebenefratelli Isola Tiberina (Rome, Italy). Exclusion criteria were: (a) medically prescribed special diets within 4 weeks prior to delivery; (b) diarrhoea or severe constipation within 2 weeks prior to delivery; (c) use of antibiotics within 2 weeks prior to delivery; (d) use of drugs affecting intestinal resorption, e.g., activated charcoal or cholestyramine, within 2 weeks prior to delivery; (e) diagnosis of a gastrointestinal pathology, e.g., ulcerative colitis or Crohn's disease, except for appendectomy; (f) cancer, HIV, or any other serious illness demanding medical treatment; (g) invasive or abrasive dental treatments within 2 weeks prior to delivery; (h) alcohol abuse (defined as Alcohol Use Disorder Identification test >10); and (i) current or recent (within the previous 4 weeks) participation in a clinical trial. Patients who decided to participate were asked to sign an informed consent form. Patients were also asked to fill in a questionnaire to record their food consumption, with a special focus on fish, shellfish, and foods employing packaging, and the use of personal care products from 7 days before the expected date of delivery to 7 days after.

2.2. Sample Collection

Breastmilk samples were collected 1 week after delivery at the Department of Obstetrics and Gynaecology of San Giovanni Calibita Fatebenefratelli Hospital (Rome, Italy). Patients were guided on a manual expression procedure, recommended by the World Health Organization and described in a document released by the Italian Ministry of Health [26], which uses manual expression to obtain the maximum milk output and to avoid pain or damage to the breast tissue. No breast pumps were allowed to avoid contamination from its plastic components. Briefly, the manual expression procedure consists of cupping the breast with one hand, with the other forming a C-shape with the thumb and the forefinger, 3–4 cm from the base of the nipple; then, pressure is applied by pushing towards the ribcage, squeezing with the thumb and forefinger, and finally releasing the pressure. The sequence of pressure, squeeze, and release was repeated until obtaining an adequate amount of milk. Milk samples were placed into glass flasks, weighed, and then stored at -20 °C until processing. Each sample contained an average amount of 4.16 ± 1.73 g of breastmilk.

2.3. Sample Digestion and Filtration

In order to remove organic components from milk samples, a digestion protocol was set up and performed at the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental Sciences, Università Politecnica delle Marche (Ancona, Italy). A 10% KOH solution prepared using 1.6 µm filtered deionised water and KOH tablets (Sigma-Aldrich) was added to each flask in a ratio of sample to KOH of 1:10 (w/v). Flasks were sealed and incubated at 40 °C for 48 h [27]. Digestates were then filtered through a 1.6 µm pore-size filter membrane (Whatman GF/A) by a vacuum pump connected to a filter funnel. Filter membranes were dried at room temperature and stored in glass Petri dishes until Raman Microspectroscopy (RMS) analysis.

2.4. Detection and Identification of MPs by Raman Microspectroscopy

RMS analysis was performed by using an XploRA Nano Raman Microspectrometer (Horiba Scientific) at the ARI Laboratory of Università Politecnica delle Marche (Ancona, Italy). All filter membranes, including those deriving from the procedural blanks, were inspected by visible light using a $\times 10$ objective (Olympus MPLAN10$\times$/0.25). The detected MPs were morphologically characterised by a $\times 100$ objective (Olympus MPLAN100$\times$/0.90) and then directly analysed on the filter by RMS (spectral range 200–1800 cm^{-1}, 532 nm or 785 nm laser diode, 600 lines per mm grating). Spectra were dispersed onto a 16-bit dynamic range Peltier-cooled CCD detector; the spectrometer was calibrated to the 520.7 cm^{-1} line of silicon prior to spectral acquisition. To reduce noise and enhance spectrum quality, raw

Raman spectra were subjected to polynomial baseline correction and vector normalisation (Labspec 6 software, Horiba Scientific). The polymer matrix of the detected particles was identified by comparing the collected Raman spectra with spectral libraries of polymers and pigments obtained by measuring standard polymers/compounds (KnowItAll software, John Wiley & Sons, Inc., Hoboken, NJ, USA) [28,29]. Similarities of more than 80 of the Hit Quality Index (HQI) were considered satisfactory.

2.5. Quality Assurance and Control (QA/QC)

Efforts were adopted to avoid microplastic contamination during sample collection, storage, processing, and analysis. To this aim, a plastic-free protocol was adopted during all phases of the experiment, and a dedicated room was used for the digestion of milk samples, filtration, and RMS analysis steps. Routinely employed plastic tools were replaced with sterilised glass ones. Cotton laboratory coats and single-use latex gloves were worn during all phases of the experiment. All liquids, including ethanol for cleaning and deionised water for cleaning and preparation of all solutions, were filtered through 1.6 μm pore-size filter membranes (Whatman GF/A). Work surfaces were thoroughly washed with 70% ethanol prior to starting all procedures and during the experimental time. Glassware and instruments, including scissors and tweezers, were washed using dishwashing liquid, triple rinsed with 70% ethanol, and finally rinsed with 1.6 μm filtered deionised water.

Moreover, environmental and procedural blanks were prepared and thoroughly analysed to detect microplastic contamination deriving from the laboratory environment and from other external sources. As regards environmental blanks, a filter membrane soaked with 1.6 μm filtered deionised water was placed into an uncovered Petri dish and positioned each day in the above-mentioned dedicated room. A procedural blank was also prepared together with every batch of samples following the exact same procedure as samples, but without adding milk. The filters deriving from environmental and procedural blanks were first inspected by stereomicroscope.

2.6. Statistical Analysis

Data analysis was performed by using the statistical software package Prism6 (Graphpad Software, Inc., San Diego, CA, USA). Normality was checked by the D'Agostino and Pearson omnibus normality test. Chi-square test, Student's *t*-test, and one-way analysis of variance (ANOVA) were performed to compare data accordingly. The significance threshold was set at $p < 0.05$.

3. Results

In the present study, N. 34 breastmilk samples were investigated by RMS for the presence of microplastics. MP contamination was found in 26 out of 34 women. As regards QA/QC protocols, the analysis of environmental (N. 14) and procedural (N. 9) blanks was performed. In the environmental blanks, only fibres, for a total of N. 16, ranging from 571 μm to 3000 μm, were found. Conversely, no MP contamination was found in the filters from the procedural blanks. Given the dimensions of the fibres, which are not compatible with the translocation into breastmilk, and the absence of fibres in the analysed milk samples, there was no need to blank-correct the results.

All details about the identified microparticles (such as morphology, dimensions, colour, polymer matrix, and pigment) are listed in Table 1.

Table 1. Information about patients (age, quantity of milk sample, and abundance of MPs) and morphological and chemical features of the identified microparticles.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#1	28	8.44	2	0.24	irregular fragment	~10 µm	orange	nitrocellulose	
					irregular fragment	~6 µm	orange	polyethylene	
#2	32	4.92	1	0.20	irregular fragment	~3 µm	blue	polyethylene	
#3	32	5.83	1	0.17	irregular fragment	~6 µm	black	polyvinyl chloride	
#4	38	5.09	2	0.39	irregular fragment	~2 µm	red	polyvinyl chloride	
					irregular fragment	~3 µm	blue	polypropylene	
#5	36	7.08	2	0.28	irregular fragment	~6 µm	red	chlorinated polyethylene	
					sphere	~5 µm	grey	polypropylene	
#6	40	1.95	4	2.05	irregular fragment	~5 µm	light blue	polyvinyl chloride	
					irregular fragment	~1 µm	blue		Pigment Blue 29 (C.I. Constitution 77007)
					irregular fragment	~10 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
					irregular fragment	~10 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
#7	38	3.49	5	1.43	sphere	~5 µm	brown	polyvinyl alcohol	
					irregular fragment	~3 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
					irregular fragment	~10 µm	brown/grey	nitrocellulose	
					sphere	~2 µm	blue		Pigment Blue 29 (C.I. Constitution 77007)
					irregular fragment	~10 µm	light blue	polypropylene	

Table 1. *Cont.*

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#8	36	4.21	2	0.47	irregular fragment	~2 μm	red		Pigment Red 101/102 (C.I. Constitution 77491)
					irregular fragment	~10 μm	red		Pigment Red 101/102 (C.I. Constitution 77491)
#9	45	2.81	3	1.07	irregular fragment	~4 μm	red		Pigment Red 101/102 (C.I. Constitution 77491)
					irregular fragment	~5 μm	yellow/orange	polyethylene	
					irregular fragment	~3 μm	light blue	polyvinyl chloride	
#10	34	5.58	2	0.36	irregular fragment	~6 μm	orange	polyethylene	
					irregular fragment	~6 μm	blue	polypropylene	
#11	39	2.32	0	0					
#12	32	3.55	2	0.56	irregular fragment	~2 μm	black	polyethylene	
					irregular fragment	~10 μm	green	poly(ethylene-co-vinyl acetate)	Pigment Green 7 (C.I. Constitution 74260
#13	41	2.76	1	0.36	irregular fragment	~12 μm	blue	polyethylene	
#14	50	4.50	3	0.67	irregular fragment	~3 μm	brown		Pigment Yellow 43 / Brown 6 (C.I. Constitution 77492)
					irregular fragment	~3 μm	light blue	polyvinyl chloride	
					sphere	~4 μm	grey	polypropylene	
#15	33	5.99	2	0.33	irregular fragment	~5 μm	orange	polyethylene	
					irregular fragment	~6 μm	red	polyvinyl chloride	

Table 1. *Cont.*

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#16	37	4.65	2	0.43	irregular fragment	~10 µm	blue	polyethylene	
					irregular fragment	~2 µm	transparent	polyethylene	
#17	32	1.64	2	1.22	sphere	~5 µm	transparent	polyethylene	
					irregular fragment	~5 µm	brown		Pigment Red 101/102 (C.I. Constitution 77491)
#18	41	3.10	1	0.32	irregular fragment	~8 µm	black	poly(ethyl methacrylate)	
#19	38	6.06	2	0.33	irregular fragment	~8 µm	orange	nitrocellulose	
					irregular fragment	~2 µm	blue/green	polypropylene	
#20	37	4.19	3	0.71	irregular fragment	~3 µm	magenta	polyvinyl chloride	
					irregular fragment	~12 µm	light blue	polyvinyl chloride	
					irregular fragment	~12 µm	green	polyvinyl chloride	
#21	40	2.36	1	0.42	irregular fragment	~5 µm	blue	acrylonitrile butadiene styrene	
#22	41	5.47	0	0					
#23	35	3.82	0	0					
#24	48	2.53	5	1.98	irregular fragment	~2 µm	orange	polystyrene	
					irregular fragment	~10 µm	yellow	polyethylene	
					irregular fragment	~12 µm	transparent	polyethylene	
					irregular fragment	~4 µm	light blue	polypropylene	
					irregular fragment	~5 µm	brown	polyester	

Table 1. *Cont.*

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#25	39	7.51	1	0.13	irregular fragment	~10 µm	blue	polyamide	
#26	37	5.65	2	0.35	irregular fragment	~6 µm	white/transparent	polyethylene	
					irregular fragment	~4 µm	light blue	polypropylene	
#27	31	3.06	1	0.33	irregular fragment	~5 µm	brown	polycarbonate	
#28	35	3.54	0	0					
#29	35	2.68	1	0.37	irregular fragment	~2 µm	light blue	polyethylene	
#30	47	1.84	5	2.72	irregular fragment	~7 µm	white/transparent	polyethylene	
					irregular fragment	~8 µm	yellow/brown	polyethylene	
					irregular fragment	~2 µm	white	polyethylene	
					irregular fragment	~4 µm	orange	high-density polyethylene	
					irregular fragment	~4 µm	blue	polyvinyl chloride	
#31	49	3.85	0	0					
#32	42	4.21	0	0					
#33	45	5.10	0	0					
#34	42	1.54	0	0					

For clarity, in Figure 1, the microphotographs and corresponding Raman spectra of some selected MPs found in the analysed samples are reported.

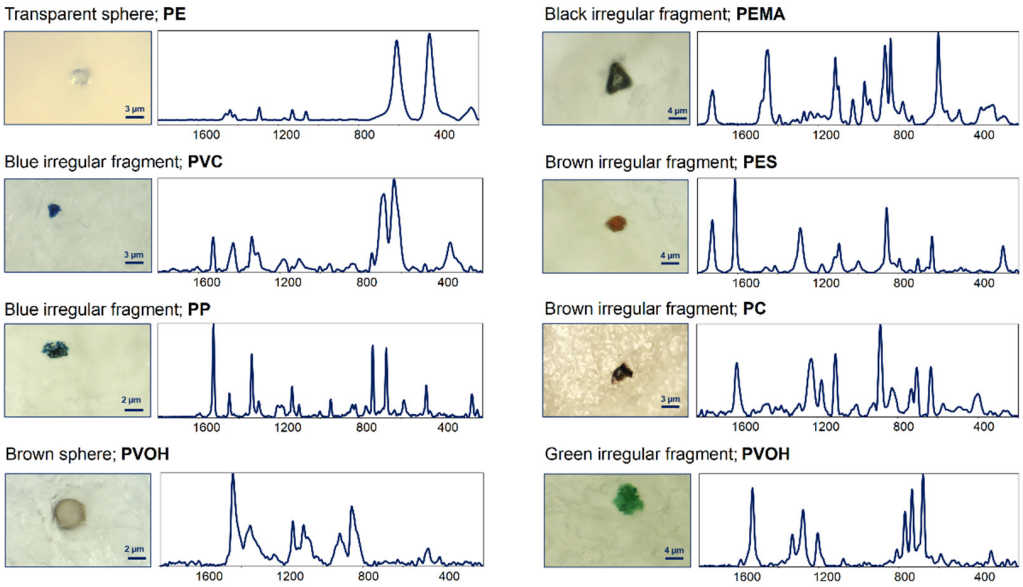

Figure 1. Microphotographs and Raman spectra (wavenumbers, cm^{-1}) of some selected MPs found in the analysed breastmilk samples. PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; PVOH: polyvinyl alcohol; PEVA: poly(ethylene-co-vinyl acetate); PEMA: poly(ethyl methacrylate); PES: polyester, and PC: polycarbonate.

The detected microparticles were classified according to their shape, colour, dimensions, and chemical composition (Figure 2). As regards the shape, only irregular fragments and spheres were found, while no films or fibres were identified (Figure 2A). Moreover, most of the identified MPs were pigmented (ca. 90%), with blue and orange/yellow being the most abundant colours (ca. 36% and ca. 17%, respectively; Figure 2B). As regards MPs' dimensions, almost half of them (ca. 47%) were in the range of 4–9 µm; ca. 29% were ≤3 µm, while ca. 24% were ≥10 µm (Figure 2C). For 48 out of the total 58 identified MPs, the polymer matrix was also identified, while for the remaining ones, the contribution of the pigments used for plastic staining was the only signal in the collected Raman spectra [21,30,31]. Within the identified polymer matrices, the most abundant ones were polyethylene (PE, 38%), polyvinyl chloride (PVC, 21%), and polypropylene (PP, 17%) (Figure 2D).

The statistical relationship between data related to patients and the presence/number of MPs was also investigated. In particular, the following patient-related parameters were analysed: patient's age; use of personal care products containing plastic compounds (including lotions, soaps, and toothpaste); and consumption, in the 7 days prior to the expected date of delivery and 7 days after, of fish/shellfish, beverages in plastic bottles, and food in plastic packaging. In Figure 3A–E, the statistical analysis of the percentages of women with and without MPs in their milk, divided according to each of the above-defined parameters, is reported. As regards 'patient's age', women were divided into three groups as follows: ≤35 years old, 36–40 years old, and ≥41 years old; no statistically significant difference was observed among groups ($p = 0.648$; Figure 3A). Moreover, women's habits of food consumption and use of personal care products were investigated, and in this case as well, no statistically significant difference among groups was observed ('fish/shellfish consumption', $p = 0.961$, Figure 3B; 'personal care product with plastics', $p = 0.1611$, Figure 3C; 'beverages in plastic bottles', $p = 0.9107$, Figure 3D; and 'food in plastic packaging', $p = 0.2963$, Figure 3E). For a deeper analysis, each of the above-defined parameters was also considered in relation to the number of detected MPs (Figure 3F–J); as expected, no significant difference among groups was revealed (one-way ANOVA or Student's *t*-test).

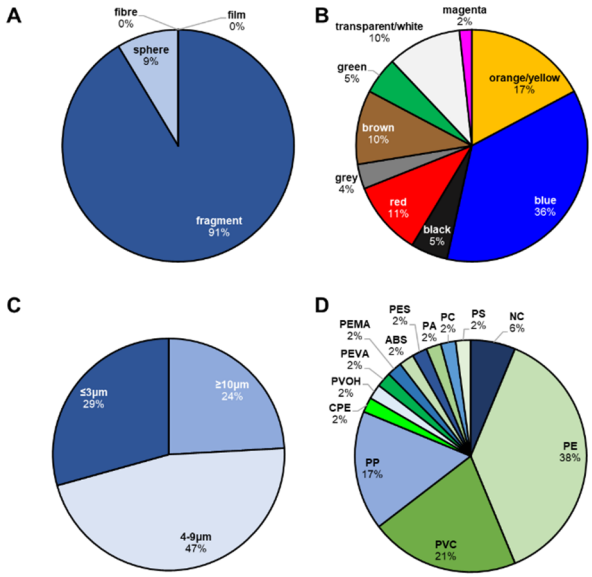

Figure 2. Percentage abundances of identified shapes (**A**), colours (**B**), dimensions (**C**), and polymer matrices (**D**). PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; CPE: chlorinated polyethylene; PVOH: polyvinyl alcohol; PEVA: poly(ethylene-co-vinyl acetate); PEMA: poly(ethyl methacrylate); ABS: acrylonitrile butadiene styrene; PES: polyester; PA: polyamide; PC: polycarbonate; PS: polystyrene, and NC: nitrocellulose.

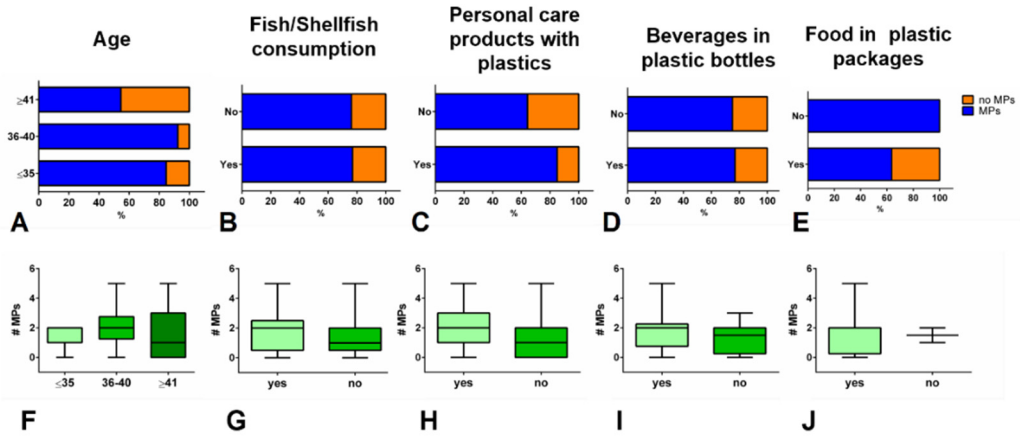

Figure 3. Percentage abundances of samples with (MPs) and without (no MPs) microplastics, divided according to the following selected parameters: (**A**) age of patient; (**B**) consumption of fish/shellfish in the 7 days prior to the expected date of delivery and 7 days after; (**C**) use of personal care products with plastic compounds in the 7 days prior to the expected date of delivery and 7 days after; (**D**) consumption of beverages in plastic bottles in the 7 days prior to the expected date of delivery and 7 days after, and (**E**) consumption of food in plastic packaging in the 7 days prior to the expected date of delivery and 7 days after. Number of identified microplastics divided according to the above-defined parameters (**F**–**J**) (box charts: centre line marks the median, edges indicate the 5th and 95th percentiles, and whiskers indicate the minimum and maximum values).

4. Discussion

Breastmilk represents the best standard nutrition for infants, thanks to its provision of nutrients and enhancement of the immune system [24]. Hence, assessing its quality in terms of possible contamination is mandatory. In fact, mothers are exposed daily to a great variety of chemicals present in the environment, for example, through food, beverages, and personal care products, and for this reason, breastmilk may be contaminated by these compounds, likely impacting children's health [32]. To date, the presence of

polychlorinated bisphenyls (PCBs), organochlorine pesticides, polybrominated diphenyl esters (PBDEs), phthalates and phthalate metabolites, per- and polyfluoroalkyl substances (PFASs), phenols, and metals have been detected in human milk [33]. In fact, since most of these contaminants are lipophilic and have a tendency to deposit in adipose tissue, they may be translocated to milk during lactation [34,35]. It is known that early life stages are the most sensitive to PCBs' toxic effects, which mainly consist of a severe impact on endocrine and cognitive systems, leading to reduced IQ and altered behaviour [36]. Similarly, PBDEs are recognised as neurotoxic, especially in children, with effects on motor, cognitive, and behavioural development [37]. Phthalates have been reported in the literature to negatively impact male reproductive functionality [38]; moreover, childhood exposure to phthalates was shown to increase the risk of allergic diseases and altered physical/neurocognitive development [39,40].

It is noteworthy that most of these environmental pollutants are also able to interact with MPs by several sorption mechanisms, which depend on polymer size, shape, density, colour, and chemical composition [41]; for example, phthalate esters have shown major sorption on polystyrene, polyethylene, and polyvinyl chloride microparticles [42]. Hence, since MPs are ubiquitous environmental contaminants and represent potential vectors for toxic organic compounds with known health impairment effects, their detection in biological matrices is actually of great concern [15–17].

In this study, for the first time, MPs were found in breastmilk samples; it should be stated that the number of microparticles that we detected could be underestimated, since only an aliquot of ~4 g of milk was considered for each sample. MPs were characterised by RMS and classified in terms of shape, dimensions, colour, and chemical composition. In this regard, almost all MPs were blue and orange/yellow irregular fragments with dimensions ranging from 2 μm to 12 μm, consistent with translocation mechanisms. In accordance with other studies reported in the literature, the most abundant polymers were polyethylene, polyvinyl chloride, and polypropylene [43,44].

Several MP routes of exposure have been reported in the literature, including inhalation, dermal contact, and ingestion, with the latter being considered the most impactful, with an estimated total intake of around 39–52 thousand per person per year [12,13]. Once ingested/inhaled, MPs can be internalised in human tissues [11]. At the gastrointestinal level, they may pass through the epithelium by endocytosis mechanisms or by paracellular diffusion, after which they are translocated by dendritic cells through the lymphatic circulation and reach the circulatory system [11]. As regards the respiratory system, inhaled MPs likely penetrate the lower respiratory tract, characterised by a thin mucus layer, and spread into the bloodstream after cellular uptake or paracellular diffusion [45].

Currently, there is growing scientific evidence about MPs in humans. Schwabl et al. reported the detection of MPs in human stool [46], while, as a further measure, Ibrahim et al. described the presence of MPs in human colectomy samples, proving that MPs in part cross the intestinal barrier [47]. As evidence of inhalation exposure, Amato-Lourenço et al. detected, in human lung tissue, <5.5 μm polymeric MPs and fibres ranging from 8.12 to 16.8 μm [48]. We recently found MPs in the human placenta, which represents the interface between the foetus and the mother exposed to the external environment [21], results that were also confirmed by Braun et al. [49]. Very recently, the presence of plastic particles in human blood finally proved the transport of MPs in the bloodstream to every body site [50].

As regards the mammary gland, two hypothetical pathways have been suggested for the translocation of exogenous particles from the bloodstream to breast milk: the mammary epithelial cell-dependent and the immune cell-dependent pathways, with the latter being particularly relevant in the case of inhaled particles [23–25,51,52]. Hence, a possible association between the presence of MPs in breastmilk and specific information regarding mothers' habits (such as the consumption of fish and shellfish, beverages in plastic bottles, and food in plastic packaging and the use of personal care products containing plastic compounds in the 7 days prior to the expected date of delivery and the 7 days after) was

investigated, but no relationship was found between MP presence/number and each of the above-mentioned parameters.

The lack of association with the use of personal care products is likely explained by considering that dermal contact has a minor impact as an exposure route, since only particles <100 nanometres can cross the dermal barrier [11]. Conversely, the absence of a relation with mothers' food habits is more difficult to explain, since the major route of MP exposure is represented by ingestion. In fact, numerous food-related sources of MPs have been reported, including fish, shellfish, and human essential daily consumables, such as table salt, sugar, bottled water, milk, honey, plastic teabags, and to a greater extent, plastic kitchen tools, plates, and packaging [53]. Hence, our findings suggest that, since MPs are ubiquitous in the environment, exposure to these microparticles is inevitable, and, for this reason, it is impossible to isolate a specific source among the complex set of faced exposures.

5. Conclusions

The evidence of MPs in human breastmilk, coupled with the previous discovery of these microparticles in the human placenta, represents a great concern, since it impacts the extremely vulnerable population of infants. In fact, the chemicals possibly contained in foods, beverages, and personal care products consumed by breastfeeding mothers may be transferred to the offspring, potentially exerting a toxic effect. Hence, it is mandatory to increase efforts in scientific research to deepen the knowledge of the potential health impairment caused by MP internalisation and accumulation, especially in infants, and to assess innovative, useful ways to reduce exposure to these contaminants during pregnancy and lactation.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee Lazio 1 (Protocol N. 708/CE Lazio 1; 24 May 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jadhav, E.B.; Sankhla, M.S.; Bhat, R.A.; Bhagat, D.S. Microplastics from food packaging: An overview of human consumption, health threats, and alternative solutions. *Environ. Nanotechnol. Monit. Manag.* **2021**, *16*, 100608. [CrossRef]
2. Lithner, D.; Larsson, Å.; Dave, G. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Sci. Total Environ.* **2011**, *409*, 3309–3324. [CrossRef] [PubMed]
3. *Plastics Europe Plastics—The Facts 2019 An Analysis of European Plastics Production, Demand and Waste Data*; Royal Society of Chemistry: Lodon, UK, 2019.
4. Eriksen, M.; Lebreton, L.C.M.; Carson, H.S.; Thiel, M.; Moore, C.J.; Borerro, J.C.; Galgani, F.; Ryan, P.G.; Reisser, J. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE* **2014**, *9*, e111913. [CrossRef]
5. Conti, I.; Simioni, C.; Varano, G.; Brenna, C.; Costanzi, E.; Neri, L.M. Legislation to limit the environmental plastic and microplastic pollution and their influence on human exposure. *Environ. Pollut.* **2021**, *288*, 117708. [CrossRef]
6. Browne, M.A.; Crump, P.; Niven, S.J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R. Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environ. Sci. Technol.* **2011**, *45*, 9175–9179. [CrossRef] [PubMed]

7. Salvador Cesa, F.; Turra, A.; Baruque-Ramos, J. Synthetic fibers as microplastics in the marine environment: A review from textile perspective with a focus on domestic washings. *Sci. Total Environ.* **2017**, *598*, 1116–1129. [CrossRef] [PubMed]
8. Hanun, J.N.; Hassan, F.; Jiang, J.-J. Occurrence, fate, and sorption behavior of contaminants of emerging concern to microplastics: Influence of the weathering/aging process. *J. Environ. Chem. Eng.* **2021**, *9*, 106290. [CrossRef]
9. Kannan, K.; Vimalkumar, K. A Review of Human Exposure to Microplastics and Insights Into Microplastics as Obesogens. *Front. Endocrinol.* **2021**, *12*, 724989. [CrossRef]
10. Sridharan, S.; Kumar, M.; Singh, L.; Bolan, N.S.; Saha, M. Microplastics as an emerging source of particulate air pollution: A critical review. *J. Hazard. Mater.* **2021**, *418*, 126245. [CrossRef]
11. Prata, J.C.; da Costa, J.P.; Lopes, I.; Duarte, A.C.; Rocha-Santos, T. Environmental exposure to microplastics: An overview on possible human health effects. *Sci. Total Environ.* **2020**, *702*, 134455. [CrossRef]
12. Prata, J.C. Airborne microplastics: Consequences to human health? *Environ. Pollut.* **2018**, *234*, 115–126. [CrossRef] [PubMed]
13. Cox, K.D.; Covernton, G.A.; Davies, H.L.; Dower, J.F.; Juanes, F.; Dudas, S.E. Human Consumption of Microplastics. *Environ. Sci. Technol.* **2019**, *53*, 7068–7074. [CrossRef] [PubMed]
14. Alimba, C.G.; Faggio, C.; Sivanesan, S.; Ogunkanmi, A.L.; Krishnamurthi, K. Micro(nano)-plastics in the environment and risk of carcinogenesis: Insight into possible mechanisms. *J. Hazard. Mater.* **2021**, *416*, 126143. [CrossRef]
15. Danopoulos, E.; Twiddy, M.; West, R.; Rotchell, J.M. A rapid review and meta-regression analyses of the toxicological impacts of microplastic exposure in human cells. *J. Hazard. Mater.* **2021**, 127861. [CrossRef]
16. Han, Y.; Lian, F.; Xiao, Z.; Gu, S.; Cao, X.; Wang, Z.; Xing, B. Potential toxicity of nanoplastics to fish and aquatic invertebrates: Current understanding, mechanistic interpretation, and meta-analysis. *J. Hazard. Mater.* **2022**, *427*, 127870. [CrossRef]
17. Yin, K.; Wang, Y.; Zhao, H.; Wang, D.; Guo, M.; Mu, M.; Liu, Y.; Nie, X.; Li, B.; Li, J.; et al. A comparative review of microplastics and nanoplastics: Toxicity hazards on digestive, reproductive and nervous system. *Sci. Total Environ.* **2021**, *774*, 145758. [CrossRef]
18. Käppler, A.; Fischer, D.; Oberbeckmann, S.; Schernewski, G.; Labrenz, M.; Eichhorn, K.-J.; Voit, B. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Anal. Bioanal. Chem.* **2016**, *408*, 8377–8391. [CrossRef]
19. Ribeiro-Claro, P.; Nolasco, M.M.; Araújo, C. Characterization of Microplastics by Raman Spectroscopy. *Compr. Anal. Chem.* **2017**, *75*, 119–151. [CrossRef]
20. Di Renzo, L.; Mascilongo, G.; Berti, M.; Bogdanović, T.; Listeš, E.; Brkljača, M.; Notarstefano, V.; Gioacchini, G.; Giorgini, E.; Olivieri, V.; et al. Potential Impact of Microplastics and Additives on the Health Status of Loggerhead Turtles (Caretta caretta) Stranded Along the Central Adriatic Coast. *Water Air Soil Pollut.* **2021**, *232*, 98. [CrossRef]
21. Ragusa, A.; Svelato, A.; Santacroce, C.; Catalano, P.; Notarstefano, V.; Carnevali, O.; Papa, F.; Rongioletti, M.C.A.; Baiocco, F.; Draghi, S.; et al. Plasticenta: First evidence of microplastics in human placenta. *Environ. Int.* **2021**, *146*, 106274. [CrossRef]
22. Eidelman, A.I.; Schanler, R.J.; Johnston, M.; Landers, S.; Noble, L.; Szucs, K.; Viehmann, L. Breastfeeding and the Use of Human Milk. *Pediatrics* **2012**, *129*, e827–e841. [CrossRef]
23. Llorca, M.; Farré, M.; Picó, Y.; Teijón, M.L.; Álvarez, J.G.; Barceló, D. Infant exposure of perfluorinated compounds: Levels in breast milk and commercial baby food. *Environ. Int.* **2010**, *36*, 584–592. [CrossRef] [PubMed]
24. LaKind, J.S.; Verner, M.-A.; Rogers, R.D.; Goeden, H.; Naiman, D.Q.; Marchitti, S.A.; Lehmann, G.M.; Hines, E.P.; Fenton, S.E. Current Breast Milk PFAS Levels in the United States and Canada: After All This Time, Why Don't We Know More? *Environ. Health Perspect.* **2022**, *130*, 025002. [CrossRef] [PubMed]
25. Jian, J.M.; Chen, D.; Han, F.J.; Guo, Y.; Zeng, L.; Lu, X.; Wang, F. A short review on human exposure to and tissue distribution of per- and polyfluoroalkyl substances (PFASs). *Sci. Total Environ.* **2018**, *636*, 1058–1069. [CrossRef]
26. Ministero della Salute Allattare al Seno-Un Investimento Per la Vita. 2019. Available online: https://www.salute.gov.it/portale/documentazione/p6_2_5_1.jsp?lingua=italiano&id=303 (accessed on 1 June 2022).
27. Karami, A.; Golieskardi, A.; Choo, C.K.; Romano, N.; Ho, Y.B.; Salamatinia, B. A high-performance protocol for extraction of microplastics in fish. *Sci. Total Environ.* **2017**, *578*, 485–494. [CrossRef]
28. Dong, M.; Zhang, Q.; Xing, X.; Chen, W.; She, Z.; Luo, Z. Raman spectra and surface changes of microplastics weathered under natural environments. *Sci. Total Environ.* **2020**, *739*, 139990. [CrossRef]
29. SLOPP Library of Microplastics. Available online: https://rochmanlab.com/slopp-and-slopp-e-raman-spectral-libraries-for-microplastics-research (accessed on 1 June 2022).
30. Imhof, H.K.; Laforsch, C.; Wiesheu, A.C.; Schmid, J.; Anger, P.M.; Niessner, R.; Ivleva, N.P. Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes. *Water Res.* **2016**, *98*, 64–74. [CrossRef]
31. Stoye, D.; Freitag, W. *Paints, Coatings and Solvents*; Wiley: Hoboken, NJ, USA, 1998; ISBN 9783527288632.
32. Karthikeyan, B.S.; Ravichandran, J.; Aparna, S.R.; Samal, A. ExHuMId: A curated resource and analysis of Exposome of Human Milk across India. *Chemosphere* **2021**, *271*, 129583. [CrossRef]
33. Lehmann, G.M.; LaKind, J.S.; Davis, M.H.; Hines, E.P.; Marchitti, S.A.; Alcala, C.; Lorber, M. Environmental Chemicals in Breast Milk and Formula: Exposure and Risk Assessment Implications. *Environ. Health Perspect.* **2018**, *126*, 096001. [CrossRef]
34. Mead, M.N. Contaminants in Human Milk: Weighing the Risks against the Benefits of Breastfeeding. *Environ. Health Perspect.* **2008**, *116*, A426–A434. [CrossRef]
35. Vasios, G.; Kosmidi, A.; Kalantzi, O.-I.; Tsantili-Kakoulidou, A.; Kavantzas, N.; Theocharis, S.; Giaginis, C. Simple physicochemical properties related with lipophilicity, polarity, molecular size and ionization status exert significant impact on the transfer of drugs and chemicals into human breast milk. *Expert Opin. Drug Metab. Toxicol.* **2016**, *12*, 1273–1278. [CrossRef] [PubMed]

36. Lundqvist, C.; Zuurbier, M.; Leijs, M.; Johansson, C.; Ceccatelli, S.; Saunders, M.; Schoeters, G.; Ten Tusscher, G.; Koppe, J. The effects of PCBs and dioxins on child health. *Acta Paediatr.* **2006**, *95*, 55–64. [CrossRef] [PubMed]
37. Gibson, E.; Siegel, E.; Eniola, F.; Herbstman, J.; Factor-Litvak, P. Effects of Polybrominated Diphenyl Ethers on Child Cognitive, Behavioral, and Motor Development. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1636. [CrossRef] [PubMed]
38. Main, K.M.; Mortensen, G.K.; Kaleva, M.M.; Boisen, K.A.; Damgaard, I.N.; Chellakooty, M.; Schmidt, I.M.; Suomi, A.-M.; Virtanen, H.E.; Petersen, J.H.; et al. Human Breast Milk Contamination with Phthalates and Alterations of Endogenous Reproductive Hormones in Infants Three Months of Age. *Environ. Health Perspect.* **2006**, *114*, 270–276. [CrossRef]
39. Braun, J.M.; Sathyanarayana, S.; Hauser, R. Phthalate exposure and children's health. *Curr. Opin. Pediatr.* **2013**, *25*, 247–254. [CrossRef]
40. Huang, H.-B.; Chen, H.-Y.; Su, P.-H.; Huang, P.-C.; Sun, C.-W.; Wang, C.-J.; Chen, H.-Y.; Hsiung, C.A.; Wang, S.-L. Fetal and Childhood Exposure to Phthalate Diesters and Cognitive Function in Children Up to 12 Years of Age: Taiwanese Maternal and Infant Cohort Study. *PLoS ONE* **2015**, *10*, e0131910. [CrossRef]
41. Rodrigues, J.P.; Duarte, A.C.; Santos-Echeandía, J.; Rocha-Santos, T. Significance of interactions between microplastics and POPs in the marine environment: A critical overview. *TrAC Trends Anal. Chem.* **2019**, *111*, 252–260. [CrossRef]
42. Liu, F.; Liu, G.; Zhu, Z.; Wang, S.; Zhao, F. Interactions between microplastics and phthalate esters as affected by microplastics characteristics and solution chemistry. *Chemosphere* **2019**, *214*, 688–694. [CrossRef]
43. Jones, J.I.; Vdovchenko, A.; Cooling, D.; Murphy, J.F.; Arnold, A.; Pretty, J.L.; Spencer, K.L.; Markus, A.A.; Vethaak, A.D.; Resmini, M. Systematic Analysis of the Relative Abundance of Polymers Occurring as Microplastics in Freshwaters and Estuaries. *Int. J. Environ. Res. Public Health* **2020**, *17*, 9304. [CrossRef]
44. Bajt, O. From plastics to microplastics and organisms. *FEBS Open Bio* **2021**, *11*, 954–966. [CrossRef]
45. Mowat, A.M. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat. Rev. Immunol.* **2003**, *3*, 331–341. [CrossRef] [PubMed]
46. Schwabl, P.; Köppel, S.; Königshofer, P.; Bucsics, T.; Trauner, M.; Reiberger, T.; Liebmann, B. Detection of Various Microplastics in Human Stool. *Ann. Intern. Med.* **2019**, *171*, 453. [CrossRef] [PubMed]
47. Ibrahim, Y.S.; Tuan Anuar, S.; Azmi, A.A.; Wan Mohd Khalik, W.M.A.; Lehata, S.; Hamzah, S.R.; Ismail, D.; Ma, Z.F.; Dzulkarnaen, A.; Zakaria, Z.; et al. Detection of microplastics in human colectomy specimens. *JGH Open* **2021**, *5*, 116–121. [CrossRef] [PubMed]
48. Amato-Lourenço, L.F.; Carvalho-Oliveira, R.; Júnior, G.R.; dos Santos Galvão, L.; Ando, R.A.; Mauad, T. Presence of airborne microplastics in human lung tissue. *J. Hazard. Mater.* **2021**, *416*, 126124. [CrossRef] [PubMed]
49. Braun, T.; Ehrlich, L.; Henrich, W.; Koeppel, S.; Lomako, I.; Schwabl, P.; Liebmann, B. Detection of Microplastic in Human Placenta and Meconium in a Clinical Setting. *Pharmaceutics* **2021**, *13*, 921. [CrossRef]
50. Leslie, H.A.; van Velzen, M.J.M.; Brandsma, S.H.; Vethaak, A.D.; Garcia-Vallejo, J.J.; Lamoree, M.H. Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* **2022**, 107199. [CrossRef]
51. Cai, J.; Zang, X.; Wu, Z.; Liu, J.; Wang, D. Translocation of transition metal oxide nanoparticles to breast milk and offspring: The necessity of bridging mother-offspring-integration toxicological assessments. *Environ. Int.* **2019**, *133*, 105153. [CrossRef]
52. Yang, L.; Kuang, H.; Zhang, W.; Wei, H.; Xu, H. Quantum dots cause acute systemic toxicity in lactating rats and growth restriction of offspring. *Nanoscale* **2018**, *10*, 11564–11577. [CrossRef]
53. Senathirajah, K.; Attwood, S.; Bhagwat, G.; Carbery, M.; Wilson, S.; Palanisami, T. Estimation of the mass of microplastics ingested—A pivotal first step towards human health risk assessment. *J. Hazard. Mater.* **2021**, *404*, 124004. [CrossRef]

Safer Choice Standard and Criteria

Safer Choice Standard | Ingredient Criteria | Product-Class Criteria

Safer Choice Standard

- **Read the most recent version of the Safer Choice Standard** <https://epa.gov/saferchoice/safer-choice-standard> **(February 2015)**
- Overview of the standard
- Changes to the standard
- Implementation and compliance schedules
- Frequently asked questions (FAQs) about ingredient disclosure, packaging, and performance requirements

Overview

The Safer Choice Standard, formerly known as DfE's Standard for Safer Products (or the "DfE Standard") identifies the requirements products and their ingredients must meet to earn the Safer Choice label.

Read the most recent version of the Safer Choice Standard <https://epa.gov/saferchoice/safer-choice-standard> (February 2015)

Changes to the Safer Choice Standard

The Safer Choice Standard was revised in February 2015. The Safer Choice Standard now includes provisions for the Safer Choice label, an associated fragrance-free label, and changes to the standard to implement the Safer Choice label.

Read the Changes to the Standard to Implement the Safer Choice Label <https://epa.gov/saferchoice/safer-choice-standard-2015-update>.

Read the Notice of Availability and Request for Comments <http://www.regulations.gov/docket/epa-hq-oppt-2015-0047>.

Implementation and compliance schedules

To ensure your products are compliant with the most recent version of the Safer Choice Standard, please read the Safer Choice Implementation and Compliance Schedules <https://epa.gov/saferchoice/safer-choice-implementation-and-compliance-schedules>.

Frequently asked questions (FAQs) about ingredient disclosure, packaging, and performance requirements

- Ingredient disclosure FAQs <https://epa.gov/sites/default/files/2015-02/documents/ingredient_disclosure_guidance.pdf>
- Packaging FAQs <https://epa.gov/sites/default/files/2015-02/documents/packaging_guidance.pdf>
- Product performance FAQs <https://epa.gov/system/files/documents/2022-04/product_performance_guidance.pdf>

Criteria for Safer Chemical Ingredients

Each chemical ingredient in a formulation has a function in making a product work - whether it is to aid in cleaning by reducing surface tension (surfactants), dissolve or suspend materials (solvents), or reduce water hardness (chelating agents). Within these "functional classes," many ingredients share similar toxicological and environmental fate characteristics. As a result, Safer Choice focuses its review of formulation ingredients on the key (environmental and human health) characteristics of concern within a functional class. This approach allows formulators to use those ingredients with the lowest hazard in their functional class, while still formulating high-performing products.

The Safer Choice Program evaluates each ingredient in a formulation against the following Master and Functional-Class Criteria documents, as appropriate. These documents define the characteristics and toxicity thresholds for ingredients that are acceptable in Safer Choice products.

The criteria are based on EPA expertise in evaluating the physical and toxicological properties of chemicals, and while they incorporate authoritative lists of chemicals of concern, they go far beyond these lists. Safer Choice applies the criteria using EPA research and analytical methods to ensure that Safer Choice products contain only the safest possible ingredients. All criteria documents are part of the Safer Choice Standard.

- Master criteria <https://epa.gov/saferchoice/safer-choice-master-criteria-safer-chemical-ingredients>
- Functional-class criteria
    - Chelating and sequestering agents <https://epa.gov/saferchoice/safer-choice-criteria-chelating-and-sequestering-agents>
    - Colorants, polymers, preservatives, and related chemicals <https://epa.gov/saferchoice/safer-choice-criteria-colorants-polymers-preservatives-and-related-chemicals>
    - Defoamers <https://epa.gov/saferchoice/safer-choice-criteria-defoamers>
    - Enzymes and enzyme stabilizers <https://epa.gov/saferchoice/safer-choice-criteria-enzymes-and-enzyme-stabilizers>
    - Fragrances <https://epa.gov/saferchoice/safer-choice-criteria-fragrances>
    - Oxidants and oxidant stabilizers <https://epa.gov/saferchoice/safer-choice-criteria-oxidants-and-oxidant-stabilizers>
    - Processing aids and additives <https://epa.gov/saferchoice/safer-choice-criteria-processing-aids-and-additives>
    - Solvents <https://epa.gov/saferchoice/safer-choice-criteria-solvents>
    - Surfactants <https://epa.gov/saferchoice/safer-choice-criteria-surfactants>

Safer Choice Product-Class Criteria

In addition to the product and ingredient criteria in the Safer Choice Standard, supplemental requirements are necessary to ensure that certain classes of products achieve best-in-class status and qualify to carry the Safer Choice label. These supplemental requirements are set forth in the criteria documents below.

- Direct release products
- Fragrance-free products
- Ice-melt products
- Inorganic- and mineral-based products
- Microorganism-based products
- Personal care products
- Safer marine lubricants
- Specialized industrial products

Criteria for environmental toxicity and fate for chemicals in direct release products

Certain products intended for use outdoors are likely to bypass sewage treatment, limiting the time for degradation prior to entering sensitive environments. For these products, like boat cleaners and graffiti removers, Safer Choice has raised the bar in its standard environmental criteria to address the potential for immediate contact with aquatic life. **Any ingredients (including surfactants, preservatives, solvents, etc.) that have aquatic toxicity values <10 mg/L are not allowed in Safer Choice direct release products.**

Supplemental criteria for chemicals in direct release products

Acute Aquatic Toxicity Value (L/E/IC50)	Chronic Aquatic Toxicity Value (LOEC)	Persistence[1] (measured in terms of rate of biodegradation)	Bioaccumulation Potential	Status
If acute aquatic	OR chronic			Not

toxicity ≤10 ppm...	toxicity ≤1 ppm...	⇒	⇒	acceptable
Acute Aquatic Toxicity Value (L/E/IC50)	**Chronic Aquatic Toxicity Value (LOEC)**	**Persistence[1] (measured in terms of rate of biodegradation)** AND	**Bioaccumulation Potential**	**Status**
If acute aquatic toxicity >10 ppm and <100 ppm...	AND chronic aquatic toxicity >1 ppm...	biodegradation[2] occurs within a 10-day window without degradation products of concern[3]...	...AND BCF/BAF <1,000[4]...	Acceptable
		...AND biodegradation[2] does not occur within a 10-day window and/or results in degradation products of concern[3]...	⇒	Not acceptable

		...AND biodegradation[2]		

Acute Aquatic Toxicity Value (L/E/IC50)	**Chronic Aquatic Toxicity Value (LOEC)**	**Persistence (measured in terms of rate of biodegradation)**	**Bioaccumulation Potential**	**Status**
If acute aquatic toxicity ≥100 ppm...	AND chronic aquatic toxicity ≥10 ppm...	...AND biodegradation[2] does not occur within 28 days and/or results in degradation products of concern[3]...	...AND BCF/BAF <1,000[4]...	Acceptable
		occurs within 28 days without [illegible] products of concern[3]...		
toxicity ≥100 ppm...	aquatic toxicity ≥10 ppm...	...AND biodegradation[2] does not occur within 28 days and/or results in degradation products of concern[3]...	⇒	Not acceptable

1. Insoluble, inert, metal-containing, or inorganic compounds may be inherently persistent and are therefore considered recalcitrant, meaning resistant to degradation or elemental. For direct release applications, recalcitrant chemicals may be acceptable if they are measured or predicted to have low aquatic toxicity; both acute (≥100 ppm) and chronic (≥10 ppm) values will be considered.

2. Generally, >60% mineralization (to CO2 and water) in a Ready Biodegradation test.

3. Products of concern are compounds with high acute or chronic aquatic toxicity (L/E/IC50 ≤ 10ppm or LOEC ≤1 ppm) and a slow rate of biodegradation (greater than 28 days).

4. Category for Persistent, Bioaccumulative and Toxic New Chemical Substance. November 4, 1999. Federal Register Notice, volume 64, issue 213.

Criteria for fragrance-free products

From Safer Choice Standard Section 3.9 (pg. 11) <https://epa.gov/saferchoice/safer-choice-standard>: "For products that qualify for the Safer Choice label, manufacturers may request an additional certification—the Fragrance-free label—to indicate that a product contains no fragrance materials. To qualify as fragrance-free, a product must only contain ingredients on or eligible for the Agency's Safer Chemical Ingredients List (SCIL) and **must not** contain any fragrance materials. Chemicals with dual functionality, i.e., that function both as a fragrance and something else, are not allowed in fragrance-free products."

Please read "Safer Choice Label – Fragrance-Free" for further details <https://epa.gov/saferchoice/safer-choice-criteria-fragrance-free-products>.

Criteria for ice-melt products

An ice-melt product under Safer Choice is, as the name implies, one that melts ice and snow at temperatures below the freezing point of water, and not simply a product that aids traction like sand. A manufacturer of a safer ice-melt product may become a Safer Choice partner provided that they agree to certain terms in their partnership agreement and that their product has the characteristics specified below. Safer Choice ice-melt products must:

1. Pass the appropriate Safer Choice Criteria.
2. Reduce sodium (Na) and chloride (Cl) use by at least 30% (under comparable use scenarios).
3. Be labeled under a Safer Choice partnership agreement in which the product manufacturer has agreed to a customer education/training plan to ensure proper product use and application rates (and reductions in Na and Cl).
4. Not contain cyanide as an anti-caking agent.
5. Function at temperatures <0 °F.
6. Comply with Pacific NW Snow Fighters' criteria <http://onlinepubs.trb.org/onlinepubs/nchrp/nchrp_rpt_577.pdf> (pdf) for reduction in corrosivity to steel (to be acceptable, a corrosion-inhibition chemical product must prove to have a percent effectiveness value of at least 70% less than Sodium Chloride).
7. Meet performance levels as evaluated under the Pacific NW Snow Fighters' criteria <http://onlinepubs.trb.org/onlinepubs/nchrp/nchrp_rpt_577.pdf> (pdf).

Criteria for inorganic- and mineral-based products

To label innovative, safer products, the Safer Choice review focuses on the evaluation of wet-chemical ingredients and formulations. Safer Choice assesses ingredients based on its safer chemical criteria and in comparison to other products/ingredients for similar uses. The Safer Choice Criteria inform on what chemistry is safer by comparing

substances, within functional use classes (surfactants, solvents, etc.), against an array of toxicological endpoints. Safer Choice allows the use of its label on those products whose ingredients derive entirely from the safer end of the human health and environmental spectrum.

The standard Safer Choice review is not oriented to evaluating a product composed solely of inorganic materials or minerals, which are typically inert and function via friction rather than chemical activity. Safer Choice recognizes, however, that these products may substitute for chemical-based products that contain ingredients of potential concern and may generate significant direct and collateral human health and environmental benefits. Safer Choice has therefore developed evaluation criteria that may make it possible to label these products (e.g., cleaners made of crushed glass or stones; **not**, however, sodium-chloride-based or similar ice-melt products for which Safer Choice has separate criteria).

A decision to allow use of the Safer Choice label will be based on the following criteria (in addition to the other applicable elements in the Safer Choice Standard):

1. **Hazard profile.**
    - The hazard profile of the inorganic or mineral materials: Ingredients must not raise any toxicological concerns. Consider, for example, if the material is asbestiform or fibrous, or if potential impurities are present (e.g., problematic metals or crystalline silica).
    - The hazard profile of the non-mineral-based ingredients: Ingredients must pass the appropriate Criteria for Safer Chemical Ingredients.
2. **Recycled content.** Product must be composed of at least 95% recycled materials. If the product includes a plastic handle or other plastic part, it must also be reusable and/or composed of at least 95% recycled content.
3. **Manufacturing/sustainability.** Candidate partner must provide information and/or data to demonstrate that only permissible air, land or water releases occur during the product's manufacturing process (e.g., via environmental release permits or waste manifests). They must also report on energy and water use as indicia of resource conservation and a baseline for continuous improvement.
4. **Safer substitution.** Product must have the potential to meet the same functional need as chemical-based products currently in use.

5. **Exposure and release.** Product in use must not generate particles that are inhalable (10 microns or less). - Product must not produce potential waste products of concern (candidate must submit an analysis of the byproducts generated).
6. **Packaging.** Packaging must comply with the Safer Choice Standard, section 4.2.6. Any paperboard in the packaging should be made of or work toward 100% recycled content.
7. **Performance.** Product must perform well in comparison to a leading brand without damaging surfaces.

Criteria for microorganism-based products

Microorganism-based products are a distinct class and subject to tailored evaluation criteria. In its review, Safer Choice carefully considers the identity and potential hazards and risks of the microbial species, as informed by its Checklist for Formulations Containing Microorganisms <https://epa.gov/saferchoice/safer-choice-criteria-formulations-containing-microorganisms>, in combination with other considerations like purity of strain, ingredient functionality and product performance, as described in its Considerations for Microorganism-based Products <https://epa.gov/saferchoice/safer-choice-criteria-formulations-containing-microorganisms>.

Please note: microbial-based products intended for use in indoor environments are not eligible for partnership. Non-microbial ingredients will be reviewed based on their respective component-class criteria.

Criteria for personal care products (PCP)

In April 2011, Safer Choice finalized section 4.5 of the Safer Choice Standard: “Products Designed for Dermal Contact.” With the increased interest in the Safer Choice label from personal care product manufacturers, the question has arisen whether all personal care products should be reviewed under section 4.5.

Safer Choice intended that the heightened requirements in section 4.5, developed in consultation with the Food and Drug Administration, would apply only to certain classes of personal care products:

- those that are “leave-on” in nature and result in prolonged dermal contact, like lotions and deodorants, and
- those that, regardless of length of exposure, are made to come into contact with infants and children, whose bodies are developing and particularly sensitive to certain chemicals.

In both cases, formulators may address the restriction on sensitizers (including ingredients that lack sensitization data) by listing them on the product label or by providing whole product sensitization testing.

The following matrix categorizes personal care products into two groups based on whether section 4.5 applies or does not apply. While Safer Choice has reviewed and labeled rinse-off personal care products, like hand soaps, Safer Choice has yet to label a leave-on product subject to section 4.5.

Section 4.5 applies (leave-on PCP)	**Section 4.5 doesn't apply (rinse-off PCP)**
Aftershave	Body wash
Astringent/toner	Bubble bath and bath salts
Cleaning wipes that don't require rinsing after use	Hair conditioner
Cuticle cream, lotion, and oil	Exfoliant products (if rinsed off)
Deodorant and antiperspirant	Face wash
Hair shine products	Hair dye, color, and bleach
Hair spray	Hair relaxants
Hair styling products (e.g., balm, gel, mousse)	Makeup remover (if rinsed off)

Section 4.5 applies (leave-on PCP)	Section 4.5 doesn't apply (rinse-off PCP)
Leave-on hair conditioner	Moisturizing products (if rinsed off)
Lip products	Nail polish remover
Makeup and bronzers (e.g., foundation, concealer, bronzer, mascara, eyeliner, eye shadow, blush)	Shampoo
Makeup remover (if left on)	Shaving cream, gel, and foam
Massage oil	Soap and cleansers
Nail polish	
Skin care products (e.g., lotion, moisturizer, cream, oil, serum)	
Sunless tanning products	

Criteria for safer marine lubricants

Manufacturers of marine lubricants subject to the Office of Water Vessel General Permit (VGP) requirements for environmentally acceptable lubricants (EALs), who wish to qualify for the Safer Choice label, must comply with the Safer Choice Standard and Criteria, with the limited exceptions and additional requirements specified below.

It is noteworthy that the chemicals in marine lubricants typically include as part of their functionality the ability to resist degradation and be effective over long periods under adverse conditions. These chemicals also can be complex molecules and mixtures and often lack measured toxicity data. To identify the safest available chemicals given their functional characteristics, the toxicity thresholds in the Safer Choice Master Criteria

<https://epa.gov/saferchoice/safer-choice-master-criteria-safer-chemical-ingredients> will be used to evaluate human health endpoints, and the thresholds below will be used for environmental endpoints.

A. **Human and environmental health requirements.** Candidate products for EAL marine lubricant status must meet, at a minimum, the following ingredient data and hazard limit requirements:

a. *For acute mammalian toxicity (section 5.1 of the Master Criteria), neurotoxicity (5.4), repeated dose toxicity (5.5), respiratory sensitization (5.7), and skin sensitization (5.8), the following data requirements apply:*

Data requirements: Screen specified R-Phrases, H-Phrases, and Authoritative Lists for each chemical present in a mixture. Chemicals with new data not yet reviewed by authoritative bodies will be subject to review

b. *For carcinogenicity (section 5.2 of the Master Criteria), genetic toxicity (5.3), and reproductive and developmental toxicity (5.6), the following data requirements apply:*

Data requirements: Screen specified R-Phrases, H-Phrases, and Authoritative Lists. All available data, measured and/or estimated, for the chemical or a suitable analog will be reviewed against the criteria using a weight-of-evidence approach.

c. *Environmental toxicity and fate*

Limitations on persistent, bioaccumulative and toxic chemicals: Acceptable chemicals must not be persistent (half-life >60 days), bioaccumulative (BCF/BAF =1,000), and aquatically toxic* (LC/EC50 <10 mg/L or NOEC/LOEC <1 mg/L).

Limitation on very persistent and very bioaccumulative chemicals: Acceptable chemicals must not be very persistent (half-life >180 days or recalcitrant) and very aquatically toxic* (LC/EC50 <1.0 mg/L or NOEC/LOEC <0.1 mg/L).

Limitation on very persistent and very toxic chemicals: Acceptable chemicals must not be very persistent (half-life >180 days or recalcitrant) and very aquatically toxic* (LC/EC50 <1.0 mg/L or NOEC/LOEC <0.1 mg/L).

Data requirements: Screen specified R-Phrases, H-Phrases, and Authoritative Lists. All available data, measured and/or estimated, for the chemical or a suitable analog will be reviewed against the criteria using a weight-of-evidence approach.

B. **Direct environmental release.** Ingredients in lubricants that are intended for use in applications that result in their immediate discharge to the environment, bypassing sewage treatment systems, must meet the Criteria for Environmental Toxicity and Fate for Chemicals in Direct Release Products, based on the biodegradation testing in OECD 306.

C. **Renewable content[1].** Products must meet the following renewable content requirements:

a. Hydraulic fluid, transmission fluid, gear oil, and grease: at least 65 percent.

b. Two-stroke oil: at least 50 percent.

D. **Performance.** Products must demonstrate acceptable performance. For example, pass the ASTM D 665 test - "Standard Test Method for Rust Preventing Characteristics of Inhibited Mineral Oil in the Presence of Water."

Criteria for specialized industrial products

Specialized Industrial Products (SIPs) are a distinct subgroup of products that meet tailored criteria under the Safer Choice Program. Safer Choice is using the term "specialized" for this subset of I/I products to distinguish them based on performance requirements from other, more common I/I products, like cleaners and detergents, and to indicate that they require certain ingredients with special, high-performance functionalities. Nevertheless, to earn the Safer Choice label, a candidate product and its ingredients must meet the general SIP criteria, in section II, as well as the subclass-specific requirements, in section III.

Read the Criteria for Specialized Industrial Products <https://epa.gov/saferchoice/safer-choice-criteria-specialized-industrial-products>.

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[1] *Renewable content* means the use of farm- or agriculture-based products, like vegetable oils and animal fats.

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Biotreatment of Industrial Effluents
2005, Pages 101-110

CHAPTER 9 - Degradation of Polymers

Mukesh Doble, Anil Kumar

https://doi.org/10.1016/B978-075067838-4/50010-5

Publisher Summary

This chapter focuses on approaches employed for the degradation of polymers. Environmental pollution by synthetic polymers, such as waste plastics and water-soluble synthetic polymers in wastewater has been recognized as a major problem. Degradation of polymers can be carried out by heat, radiation, or biochemical treatment. The radiant energy may be high-energy radiation from gamma rays, ion beams, and electrons or even low-energy radiation from ultraviolet (UV) light. UV stabilizers added to polymer products reduce the rate of degradation. Chemical degradation results from treatment with chemicals such as acids and alkalis. Biodegradation of polymers results from the use of microorganisms and enzymes. Since most of the polymers are resistant to degradation, research over the past several years has focused on developing biodegradable polymers that are degraded and ultimately catabolized to carbon dioxide and water by bacteria and fungi under natural conditions. During the degradation process, they should not generate any substances that are harmful. These polymers can be classified into three major categories: polyesters produced by microorganisms, natural polysaccharides and other biopolymers like starch, and synthetic polymers like aliphatic polymers. Another approach toward achieving biodegradability has been through the addition of biodegradable groups into the main chain during the production of industrial polymers prepared by free radical copolymerization. Two such approaches are the use of ethylene bis(mercaptoacetate) as a chain transfer agent during the copolymerization of styrene and MMA, and the preparation of copolymers of vinylic monomers with cyclic comonomers containing the biodegradable functions such as ketene acetal and cyclic disulfides.

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Plastic pollution is growing relentlessly as waste management and recycling fall short, says OECD

22/02/2022 - The world is producing twice as much plastic waste as two decades ago, with the bulk of it ending up in landfill, incinerated or leaking into the environment, and only 9% successfully recycled, according to a new OECD report.

Ahead of UN talks on international action to reduce plastic waste, the OECD's first **Global Plastics Outlook** shows that as rising populations and incomes drive a relentless increase in the amount of plastic being used and thrown away, policies to curb its leakage into the environment are falling short.

Almost half of all plastic waste is generated in OECD countries, according to the Outlook. Plastic waste generated annually per person varies from 221 kg in the United States and 114 kg in European OECD countries to 69 kg, on average, for Japan and Korea. Most plastic pollution comes from inadequate collection and disposal of larger plastic debris known as macroplastics, but leakage of microplastics (synthetic polymers smaller than 5 mm in diameter) from things like industrial plastic pellets, synthetic textiles, road markings and tyre wear are also a serious concern.

OECD countries are behind 14% of overall plastic leakage. Within that, OECD countries account for 11% of macroplastics leakage and 35% of microplastics leakage. The Outlook notes that international co-operation on reducing plastic pollution should include supporting lower-income countries in developing better waste management infrastructure to reduce their plastic leakage.

The report finds that the COVID-19 crisis led to a 2.2% decrease in plastics use in 2020 as economic activity slowed, but a rise in littering, food takeaway packaging and plastic medical equipment such as masks has driven up littering. As economic activity resumed in 2021, plastics consumption has also rebounded.

Reducing pollution from plastics will require action, and international co-operation, to reduce plastic production, including through innovation, better product design and developing environmentally friendly alternatives, as well as efforts to improve waste management and increase recycling.

Bans and taxes on single-use plastics exist in more than 120 countries but are not doing enough to reduce overall pollution. Most regulations are limited to items like plastic bags, which make up a tiny share of plastic waste, and are more effective at reducing littering than curbing plastics consumption. Landfill and incineration taxes that incentivise recycling only exist in a minority of countries. The Outlook calls for greater use of instruments such as Extended Producer Responsibility schemes for packaging and durables, landfill taxes, deposit-refund and Pay-as-You-Throw systems.

Most plastics in use today are virgin – or primary – plastics, made from crude oil or gas. Global production of plastics from recycled – or secondary – plastics has more than quadrupled from 6.8 million tonnes (Mt) in 2000 to 29.1 Mt in 2019, but this is still only 6% of the size of total plastics production. More needs to be done to create a separate and well-functioning market for recycled plastics, which are still viewed as substitutes for virgin plastic. Setting recycled content targets and investing in improved recycling technologies could help to make secondary markets more competitive and profitable.

Some key findings from the Outlook:

- Plastic consumption has quadrupled over the past 30 years, driven by growth in emerging markets. Global plastics production doubled from 2000 to 2019 to reach 460 million tonnes. Plastics account for 3.4% of global greenhouse gas emissions.
- Global plastic waste generation more than doubled from 2000 to 2019 to 353 million tonnes. Nearly two-thirds of plastic waste comes from plastics with lifetimes of under five years, with 40% coming from packaging, 12% from consumer goods and 11% from clothing and textiles.

- Only 9% of plastic waste is recycled (15% is collected for recycling but 40% of that is disposed of as residues). Another 19% is incinerated, 50% ends up in landfill and 22% evades waste management systems and goes into uncontrolled dumpsites, is burned in open pits or ends up in terrestrial or aquatic environments, especially in poorer countries.

- In 2019, 6.1 million tonnes (Mt) of plastic waste leaked into aquatic environments and 1.7 Mt flowed into oceans. There is now an estimated 30 Mt of plastic waste in seas and oceans, and a further 109 Mt has accumulated in rivers. The build-up of plastics in rivers implies that leakage into the ocean will continue for decades to come, even if mismanaged plastic waste could be significantly reduced.

- Considering global value chains and trade in plastics, aligning design approaches and the regulation of chemicals will be key to improving the circularity of plastics. An international approach to waste management should lead to all available sources of financing, including development aid, being mobilised to help low and middle-income countries meet estimated costs of EUR 25 billion a year to improve waste management infrastructure.

Access the **OECD Global Plastics Outlook Database**

Watch a presentation of the report: **Green Talk Live**

Read a summary of key messages: **Global Plastics Outlook: Policy Highlights**

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Also Available

廃棄物の管理とリサイクルが不十分で、プラスチック汚染の拡大が止まらない

La contaminación por plástico crece sin cesar, en tanto que la gestión de residuos y el reciclaje se quedan cortos, dice la OCDE.

Microbeads are tiny pieces of polyethylene plastic added to health and beauty products, such as some cleansers and toothpastes.

As an emerging field of study, not a lot is known about microplastics and their impacts yet. The NOAA Marine Debris Program is leading efforts within NOAA to research this topic. Standardized field methods for collecting sediment, sand, and surface-water microplastic samples have been developed and continue to undergo testing. Eventually, field and laboratory protocols will allow for global comparisons of the amount of microplastics released into the environment, which is the first step in determining the final distribution, impacts, and fate of this debris.

Microplastics come from a variety of sources, including from larger plastic debris that degrades into smaller and smaller pieces. In addition, microbeads, a type of microplastic, are very tiny pieces of manufactured polyethylene plastic that are added as exfoliants to health and beauty products, such as some cleansers and toothpastes. These tiny particles easily pass through water filtration systems and end up in the ocean and Great Lakes, posing a potential threat to aquatic life.

Microbeads are not a recent problem. According to the United Nations Environment Programme, plastic microbeads first appeared in personal care products about fifty years ago, with plastics increasingly replacing natural ingredients. As recently as 2012, this issue was still relatively unknown, with an abundance of products containing plastic microbeads on the market and not a lot of awareness on the part of consumers.

On December 28, 2015, President Obama signed the Microbead-Free Waters Act of 2015 (https://www.gpo.gov/fdsys/pkg/BILLS-114hr1321enr/pdf/BILLS-114hr1321enr.pdf), banning plastic microbeads in cosmetics and personal care products.

Video Transcript

Plastic is everywhere. A lot of it ends up in the ocean. Most plastics in the ocean break up into very small particles. These small plastic bits are called "microplastics." Other plastics are intentionally designed to be small. They're called microbeads and are used in many health and beauty products. They pass unchanged through waterways into

the ocean. Aquatic life and birds can mistake microplastics for food. Research is being conducted. But there's still much we don't know. In 2015, the U.S. banned the use of microbeads. But microplastics are still a huge problem. You can help keep plastic out of the ocean. Remember: Reduce. Reuse. Recycle.

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Plasticenta: First evidence of microplastics in human placenta

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ABSTRACT

Microplastics are particles smaller than five millimeters deriving from the degradation of plastic objects present in the environment. Microplastics can move from the environment to living organisms, including mammals. In this study, six human placentas, collected from consenting women with physiological pregnancies, were analyzed by Raman Microspectroscopy to evaluate the presence of microplastics. In total, 12 microplastic fragments (ranging from 5 to 10 μm in size), with spheric or irregular shape were found in 4 placentas (5 in the fetal side, 4 in the maternal side and 3 in the chorioamniotic membranes); all microplastics particles were characterized in terms of morphology and chemical composition. All of them were pigmented; three were identified as stained polypropylene a thermoplastic polymer, while for the other nine it was possible to identify only the pigments, which were all used for man-made coatings, paints, adhesives, plasters, finger paints, polymers and cosmetics and personal care products.

1. Introduction

In the last century, the global production of plastics has reached 320 million tons (Mt) per year, and over 40% is used as single-use packaging, hence producing plastic waste. In Europe, plastic production reached the 58 millions of tons in 2014 (PlasticsEurope, 2016). The degradation that plastics undergo when released into the environment is a serious issue. Atmospheric agents, such as waves, abrasion, ultraviolet radiation and photo-oxidation in combination with bacteria degrade plastic fragments into micro and nanosized particles. Most of the seabed all over the world and in the Mediterranean Sea in particular, is made of plastic, resulting from the waste collected on the coasts and in the sea (de Souza Machado et al., 2018). Microplastics (MPs) are defined as particles less than 5 mm in size (Hartmann et al., 2019). MPs do not derive only from larger pieces fragmentation but are also produced in these dimensions for commercial uses. Furthermore, there are several reports of MPs in food (Barboza et al., 2018), and in particular in seafood, sea salt (Karami et al., 2017b; Kosuth et al., 2018), and in drinking water (Schymanski et al., 2018). Microplastics have also been detected in the gastrointestinal tract of marine animals (Deng et al., 2017; Reineke et al., 2013), and also human intestine (Schwabl et al., 2019). Inside tissues, MPs are considered as foreign bodies by the host organism and, as such, trigger local immunoreactions. Furthermore, MPs can act as carriers for other chemicals, such as environmental pollutants and plastic additives, which may be released and are known for their harmful effects (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016; Wright and Kelly, 2017).

In this study, for the first time, several microplastic fragments were detected by Raman Microspectroscopy in human placenta samples collected from six consenting patients with uneventful pregnancies. Raman Microspectroscopy is a well-assessed vibrational technique, widely and successfully applied in the biomedical field, to characterize both biological samples (Notarstefano et al., 2020, 2019), and to detect the occurrence of microplastics and microparticles in general (Käppler et al., 2016; Ribeiro-Claro et al., 2017). Placenta finely regulates foetal to maternal environment and, indirectly, to the external one, acting as a

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crucial interface via different complex mechanisms (PrabhuDas et al., 2015). The potential presence of man-made MPs in this organ may harm the delicate response of differentiation between self and non-self (Nancy et al., 2012) with a series of related consequences on embryo development that need to be defined.

2. Materials and methods

2.1. Experimental design

This was a pilot observational descriptive preclinical study, with a prospective and unicentric open cohort. It was approved by the Ethical Committee Lazio 1 (Protocol N. 352/CE Lazio 1; March 31th, 2020), and it was carried out in full accordance with ethical principles, including The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. To participate to this study, six selected consenting patients signed an informed consent, which included donation of placentas.

To prevent plastic contamination, a plastic-free protocol was adopted during the entire experiment. Obstetricians and midwives used cotton gloves to assist women in labour. In the delivery room, only cotton towels were used to cover patients' beds; graduate bags to estimate postpartum blood loss were not used during delivery, but they were brought in the delivery room only after birth, when umbilical cord was already clamped and cut with metal clippers, avoiding contact with plastic material. Pathologists wore cotton gloves and used metal scalpels.

The schematic illustration for the overall concept and experimental procedure is reported in Fig. 1.

2.2. Enrolment of patients and placentas collection

All recruited women were healthy and have a vaginal delivery at term of pregnancy at the Department of Obstetrics and Gynaecology of San Giovanni Calibita Fatebenefratelli Hospital, Isola Tiberina, Roma (Italy). They were selected according to the following exclusion criteria: diagnosis of gastrointestinal disease, such as ulcerative colitis, or Crohn's disease, cancer, organ transplantation, HIV (Human Immunodeficiency Virus), or other severe pathologies; alcohol abuse (defined as a >10 score in the Alcohol Use Disorders Identification Test); cigarette smoking; peculiar diets prescribed for particular medical conditions (four weeks before delivery); diarrhoea or constipation (two weeks before delivery); antibiotics intake (two weeks before delivery); assumption of drugs affecting intestinal reabsorption, such as activated charcoal, or cholestyramine (two weeks before delivery); invasive or abrasive dental treatments (two weeks before delivery); participation to

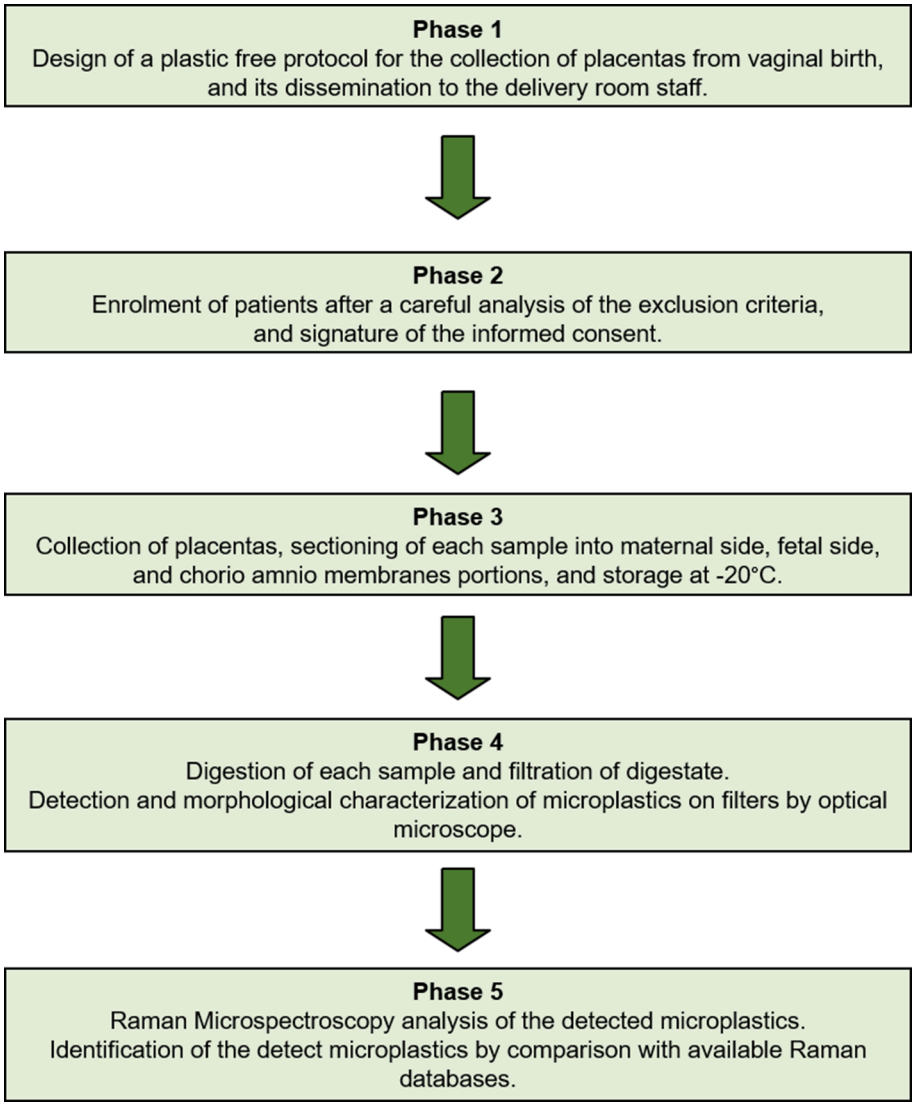


Fig. 1. Schematic illustration for the overall concept and experimental procedure followed in the study.

a clinical study (four weeks before delivery). Women were also asked to fill a questionnaire to record their food consumption (omnivorous, vegetarian, vegan, with no diet restriction) the week before delivery and the use of toothpastes and cosmetics containing MPs or synthetic polymers.

After birth, placentas were deposed onto a metal container and immediately sectioned in portions (mean weight: 23.3 ± 5.7 g) taken from maternal side, foetal side, and chorioamniotic membranes. All samples were strictly anonymous; they were labelled with number codes and stored in glass bottles with metal lids at −20 °C with no further treatment.

2.3. Digestion of placenta samples

The digestion of placenta samples was performed at the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental Sciences, Università Politecnica delle Marche (Ancona, Italy), modifying as follows the protocols from two previous works (Dehaut et al., 2016; Karami et al., 2017a). Samples were weighed and placed in a glass container. A 10% KOH solution was prepared using 1.6 μm-filtered deionised water and KOH tablets (Sigma-Aldrich). This solution was added to each jar in a ratio with the sample of 1:8 (w/v). The containers were then sealed and incubated at room temperature for 7 days. To prevent plastic contamination, cotton laboratory coats, face masks and single-use latex gloves were worn during sample handling, preparation of samples and during the entire experiment. Work surfaces were thoroughly washed with 70% ethanol prior starting all procedures. All liquids (deionised water for cleaning and for preparation of KOH solution) were filtered through 1.6 μm-pore-size filter membrane (Whatman GF/A). Glassware and instruments, including scissors, tweezers and scalpels, were washed using dishwashing liquid, rinsed with deionised water and finally rinsed with 1.6 μm-filtered deionised water. Since the experiments were conducted without the use of the laminar flow hood, the plastic fibres found in the samples were not considered in the results. Digestates were then filtered through 1.6 μm-pore-size filter membrane (Whatman GF/A) using a vacuum pump connected to a filter funnel. The filter papers were dried at room temperature and stored in glass Petri dishes until visual identification and spectroscopic characterization of plastic particles. Three procedural blanks, obtained following the same procedure above described, but without placenta samples and maintained close to the samples during their manipulation, were tested to monitor and correct potential contaminations (Karami et al., 2017a).

2.4. Analysis of microplastics by Raman Microspectroscopy

The Raman analysis of MPs was performed at the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental Sciences, Università Politecnica delle Marche (Ancona, Italy). Filter membranes were first inspected by visible light using a ×10 objective (Olympus MPLAN10x/0.25). The detected MPs were morphologically characterized by a ×100 objective (Olympus MPLAN100x/0.90), and then directly analyzed on the filter by Raman Microspectroscopy (spectral range 160–2000 cm^{-1}, 785 nm laser diode, 600 lines per mm grating). A Raman XploRA Nano Microspectrometer (Horiba Scientific) was used. The spectra were dispersed onto a 16-bit dynamic range Peltier cooled CCD detector; the spectrometer was calibrated to the 520.7 cm^{-1} line of silicon prior to spectral acquisition. Raw Raman spectra were submitted to polynomial baseline correction and vector normalization, in order to reduce noise and enhance spectrum quality (Labspec 6 software, Horiba Scientific). The collected Raman spectra were compared with those reported in the SLOPP Library of Microplastics ("SLOPP Library of Microplastics," n.d.) and in the spectral library of the KnowItAll software (Bio-Rad Laboratories, Inc.). Similarities of more than 80 of Hit Quality Index (HQI) were considered satisfactory.

3. Results

From each placenta, three portions with a mean weight of 23.3 ± 5.7 g were collected from the maternal side, the foetal side and the chorioamniotic membranes. All portions were opportunely processed for the subsequent analysis by Raman Microspectroscopy.

In total, 12 MP fragments (named #1–#12) were detected in the placentas of four women; more in detail, 5 MPs were found in the foetal side portions, 4 in the maternal side portions, and 3 in the chorioamniotic membranes. Microplastics #1–#4, #6, #7, and #9–#12, were ~10 μm in size, while #5 and #8 ones were smaller (~5 μm). All the analyzed MPs were pigmented.

A retrospective analysis based on Raman spectral information and data reported in literature was performed to define the nature of these MPs. Firstly, the collected Raman spectra were compared with those stored in the spectral library of the KnowItAll software (Bio-Rad Laboratories, Inc.). In many cases, the collected Raman spectra showed, above all, the contribution of the pigments used for plastic staining (Imhof et al., 2016; Stoye and Freitag, 1998); it is known that the conjugated rings present in pigment molecules are highly polarizable and, hence, their Raman signals are more intense than those of the apolar polymeric matrix (Käppler et al., 2016). In these cases, the KnowItAll software identified the pigments contained in the MPs. By matching the results from the KnowItAll software with the information obtained by consulting the European Chemical Agency, ECHA ("European Chemical Agency," n.d.), it was possible to accurately identify the commercial name, chemical formula, IUPAC name and Color Index Constitution Number of all pigments. Further, in order to uncover the identity of the polymer matrix of the detected MPs, the collected Raman spectra were also compared with those reported in the SLOPP library of Microplastics.

The identified MPs were differentiated between stained MPs (particles #2, #10 and #11, identified as polypropylene) and paint/coating/dye MPs (particles #1, #3–9, and #12), which are applied for paints, coatings, adhesives, plasters, polymers and cosmetics and personal care products (Imhof et al., 2016).

The microphotographs and the Raman spectra of all analysed MPs are shown in Fig. 2, while Table 1 reports their morphological and chemical characterization. The spectral analysis is reported below.

Particle #1 (Fig. 2a): the Raman spectrum resulted superimposable to the one of the pigment Iron hydroxide oxide yellow (main peak at 396 cm^{-1}, related to the vibrations of iron oxides/hydroxides) (Sklute et al., 2018).

Particles #2, #5, and #10 (Fig. 2b, e): the Raman spectra resulted comparable to the one of a polypropylene (PP) blue sample, sharing the main peaks at 253 cm^{-1} (wagging of CH_2 moieties, bending of CH moieties), 397 cm^{-1} (wagging of CH_2 moieties, bending of CH moieties), 839 cm^{-1} (rocking of CH_2 and CH_3 moieties, stretching of CC and C-CH_3 moieties), 970 cm^{-1} (rocking of CH_3 moieties), and 1455 cm^{-1} (bending of CH_3 and CH_2 moieties), all assigned to PP (Andreassen, 1999). The bands at 679 cm^{-1}, 1143 cm^{-1}, 1340 cm^{-1} and 1527 cm^{-1}, common to reference blue polypropylene and sample spectra, are known to be related to Raman signals of blue pigments, mainly based on copper phthalocyanine (Aguayo et al., 2010; Scherrer et al., 2009).

Particle #3 (Fig. 2c): the Raman spectrum resulted superimposable to the one of the blue pigment phthalocyanine, sharing the main peaks at 679 cm^{-1}, 1143 cm^{-1}, 1340 cm^{-1} and 1527 cm^{-1} (Aguayo et al., 2010; Scherrer et al., 2009).

Particle #4 (Fig. 2d): the Raman spectrum resulted superimposable to the one of the pigment violanthrone, with the two main peaks centered at 1577 cm^{-1} (C-C stretching of benzene ring) and 1307 cm^{-1} (in reference spectrum, an additional shoulder at ~1350 cm^{-1} is visible, assigned to C-C stretching and HC-C bending) (Socrates, 2001).

Particles #6 and #7 (Fig. 2f): the Raman spectra resulted superimposable to the one of the red pigment oxo(oxoferriooxy)iron (main peaks at 220, 287 and 401 cm^{-1}, typical of iron oxides) (Testa-Anta et al., 2019).

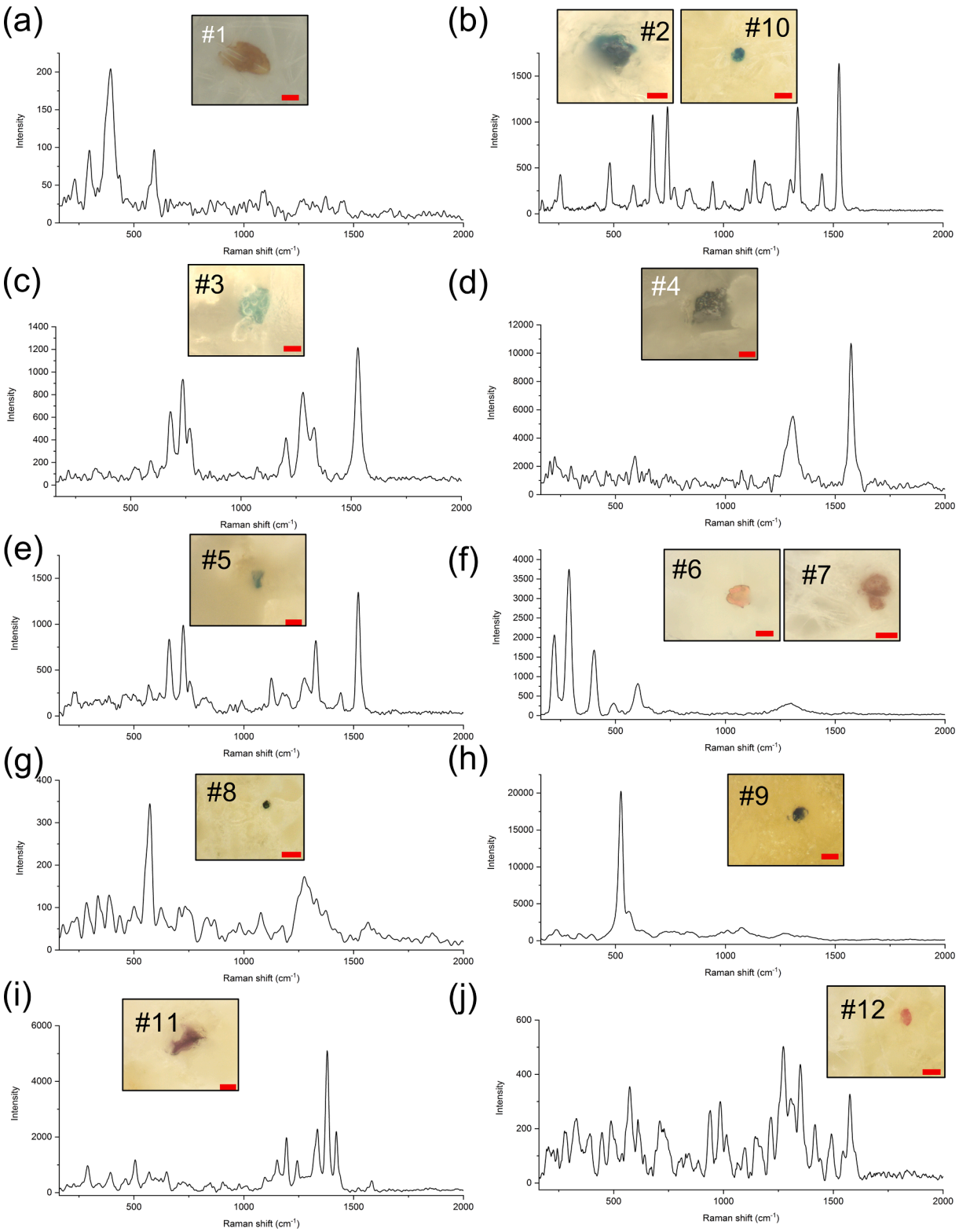


Fig. 2. Microphotographs and Raman spectra of the microplastics found in human placenta: (a) Particle #1 (scale bar 5 μm); (b) Particles #2 and #10 (scale bar 5 μm for #2 and 10 μm for #10); (c) Particle #3 (scale bar 5 μm); (d) Particle #4 (scale bar 5 μm); (e) Particle #5 (scale bar 5 μm); (f) Particles #6 and #7 (scale bar 10 μm for #6 and 5 μm for #7); (g) Particle #8 (scale bar 10 μm); (h) Particle #9 (scale bar 10 μm); (i) Particle #11 (scale bar 5 μm), and (l) Particle #12 (scale bar 10 μm).

Particle #8 (Fig. 2g): the Raman spectrum resulted superimposable to the one of the pigment Direct Blue 80, showing two main peaks at 578 cm^{-1} and 1275 cm^{-1} (both assigned to ring deformations of substituted benzene) (Socrates, 2001).

Particle #9 (Fig. 2h): the Raman spectrum resulted superimposable to the one of the pigment Ultramarine Blue, with one main peak centred at 525 cm^{-1}, assigne to orthosilicate vibration modes (Socrates, 2001).

Particle #11 (Fig. 2i): the Raman spectrum resulted comparable to the one of a PP purple fiber, sharing the main peaks of PP (Andreassen, 1999) (397 cm^{-1}, assigned to the wagging of CH_2 moieties/bending of

Table 1
Size, color and chemical features of the detected microplastics and relative pigments, together with information regarding the placenta portion in which they were found (fetal side FS; maternal side MS, and chorioamnio membrane CAM; not defined n.d.; Hit Quality Index HQI).

Particle	Placenta Portion	Microparticles			Pigment		
		Size	Color	Polymer matrix	Generic name	Molecular formula and IUPAC name	HQI
#1	FS	~10 μm	Orange	n.d.	Iron hydroxide oxide yellow (Pigment Yellow 43; C.I. Constitution 77492)	FeO(OH) iron(III) oxide hydroxide	89.97
#2	CAM	~10 μm	Blue	Polypropylene	Copper phthalocyanine (Pigment Blue 15; C.I. Constitution 74160)	$C_{32}H_{16}CuN_8$ (29H,31H-phthalocyaninato(2 −)-N29,N30,N31,N32)copper(II)	82.86
#3	FS	~10 μm	Blue	n.d.	Phthalocyanine Blue BN (Pigment Blue 16; C.I. Constitution 74100)	$C_{32}H_{18}N_8$29H,31H-phthalocyanine	89.16
#4	MS	~10 μm	Dark blue	n.d.	Violanthrone (Pigment Blue 65; C. I. Constitution 59800)	$C_{34}H_{16}O_2$Anthra[9,1,2-cde]benzo[rst]pentaphene-5,10-dione	86.44
#5	MS	~5 μm	Blue	Polypropylene	Copper phthalocyanine (Pigment Blue 15; C.I. Constitution 74160)	$C_{32}H_{16}CuN_8$ (29H,31H-phthalocyaninato(2−)-N29,N30,N31, N32)copper(II)	86.15
#6	MS	~10 μm	Red	n.d.	Diiron trioxide (Pigment Red 101/102; C.I. Constitution 77491)	Fe_2O_3 Oxo(oxoferriooxy)iron	83.65
#7	MS	~10 μm	Red	n.d.	Diiron trioxide (Pigment Red 101/102; C.I. Constitution 77491)	Fe_2O_3Oxo(oxoferriooxy)iron	89.80
#8	CAM	~5 μm	Dark blue	n.d.	Pigment Direct Blue 80	$C_{32}H_{14}Cu_2N_4Na_4O_{16}S_4$ Dicopper,tetrasodium,3-oxido-4-[[2-oxido-4-[3-oxido-4-[(2-oxido-3,6-disulfonatonaphthalen-1-yl)diazenyl] phenyl]phenyl] diazenyl]naphthalene-2,7-disulfonate	84.55
#9	CAM	~10 μm	Dark blue	n.d.	Ultramarine Blue (Pigment Blue 29; C.I. Constitution 77007)	$Al_6Na_8O_{24}S_3Si_6$ Aluminium Sodium orthosilicate trisulfane-1,3-diide	91.96
#10	FS	~10 μm	Blue	Polypropylene	Copper phthalocyanine (Pigment Blue 15; C.I. Constitution 74160)	$C_{32}H_{16}CuN_8$ (29H,31H-phthalocyaninato(2−)-N29,N30,N31, N32)copper(II)	80.60
#11	FS	~10 μm	Violet	Polypropylene	Hostopen violet (Pigment Violet 23; C.I. Constitution 51319)	$C_{34}H_{22}Cl_2N_4O_2$ 8,18-Dichloro-5,15-diethyl-5,15-dihydrodiindolo(3,2-b:3′,2′-m) tri- phenodioxazine	80.92
#12	FS	~10 μm	Pink	n.d.	Novoperm Bordeaux HF3R (Pigment Violet 32; C.I. Constitution 12517)	$C_{27}H_{24}N_6O_7S$ 4-[(E)-2-[2,5-dimethoxy-4-(methylsulfamoyl)phenyl]diazen-1-yl]-3-hydroxy-N-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl) naphthalene-2-carboxamide	84.57

CH moieties, and 1455 cm^{-1}, assigned to the bending of CH_3 and CH_2 moieties), and also of the violet pigment (1193 cm^{-1}, 1335 cm^{-1} and 1381 cm^{-1}) (Scherrer et al., 2009).

Particle #12 (Fig. 2j): the collected Raman spectrum resulted superimposable to the one of the pink pigment Novoperm Bordeaux HF3R. The Raman spectrum of this monoazopigment shared with the sample spectrum the main peaks centered at 731 cm^{-1}, 961 cm^{-1}, 1219 cm^{-1}, 1280 cm^{-1}, 1360 cm^{-1}, and 1580 cm^{-1}. This pigment is reported to be used to permanently coat and protect wood surfaces, in photographic chemicals, inks and toners, given its high solvent resistance and good heat stability (Scherrer et al., 2009).

4. Discussion

This is the first study revealing the presence of pigmented microplastics and, in general, of man-made particles in human placenta. The presence of pigments in all analysed MPs is explained by the wide use of these compounds to colour not only plastic products, but also paints and coatings, which are as ubiquitous as MPs (Imhof et al., 2016). For example, the pigment Iron hydroxide oxide yellow (particle #1) is used for coloration of polymers (plastics and rubber) and in a wide variety of cosmetics, such as BB creams and foundations; copper phthalocyanine (particles #2, #5, #10,) and phthalocyanine (particle #3) are used for staining of plastic materials (polyvinylchloride, low density polyethylene, high density polyethylene, polypropylene, polyethylene terephthalate), and for finger paints; the pigment violanthrone (particle #4) is used especially for textile (cotton/polyester) dyeing, coating products, adhesives, fragrances and air fresheners; the pigment Ultramarine blue is mainly applied in cosmetics, for example for formulations of soap, lipstick, mascara, eye shadow and other make-up products.

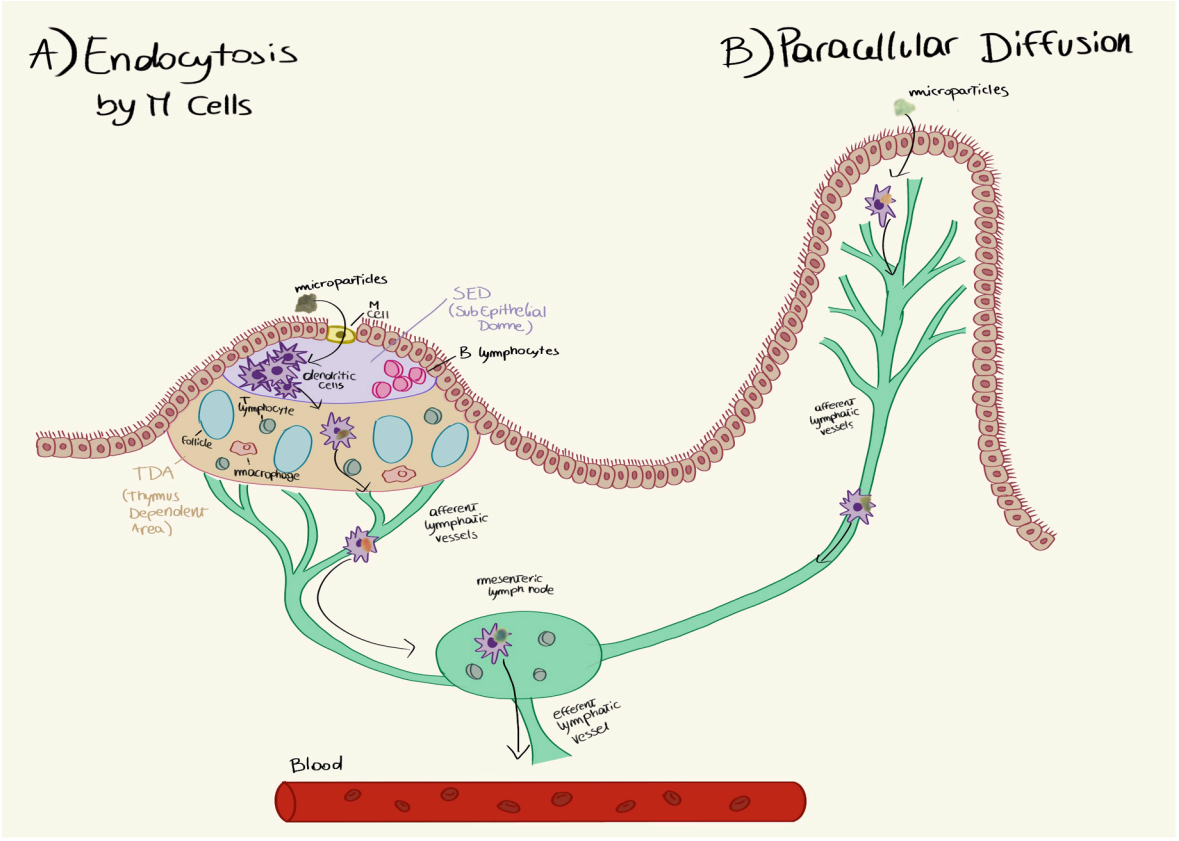
For the first time, by means of Raman Microspectroscopy, 12 MP fragments were isolated in four human placentas. In particular, 5 MPs were found in the foetal side, 4 in the maternal side and 3 in the chorioamniotic membranes, indicating that these MPs, once inside the human body, can reach placenta tissues at all levels. It is noteworthy to remark that small portions of placentas (~23 g with respect to a total weight of ~600 g) were analysed, letting hypothesize that the number of MPs within the entire placenta is much higher.

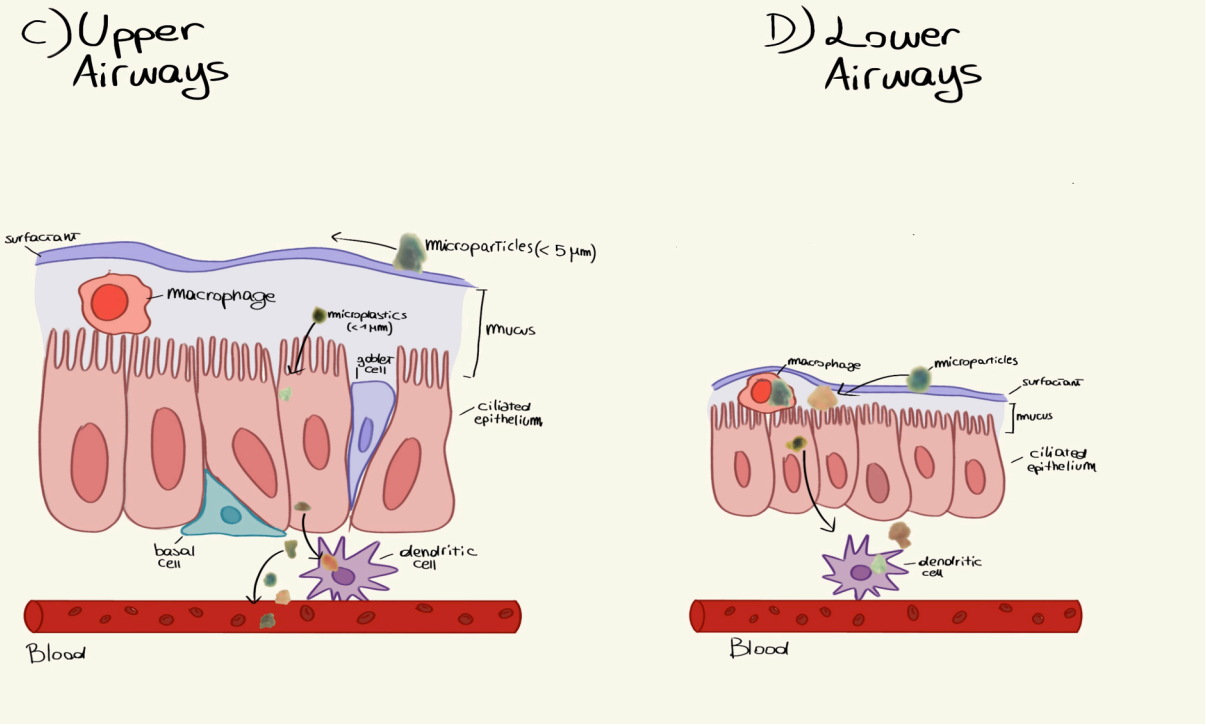
The dimensions of all MPs were ~10 μm in size, except for two that were smaller (~5 μm). These values are compatible with a possible transportation by bloodstream. In fact, previous analyses performed by means of Electron Microscopy coupled with an X-ray microprobe, revealed the presence of 5–10 μm particles as foreign bodies in human internal organs (Vaseashta, 2015).

Unfortunately, we do not know how MPs reach the bloodstream and if they come from the respiratory system or the gastrointestinal system. Fig. 3 shows the possible ways of entry and transport of the MPs from the respiratory and gastric organs to the placenta.

The presence of MPs in the placenta tissue requires the reconsideration of the immunological mechanism of self-tolerance. Placenta represents the interface between the foetus and the environment (PrabhuDas et al., 2015). Embryos and foetuses must continuously adapt to the maternal environment and, indirectly, to the external one, by a series of complex responses. An important part of this series of responses consists in the ability to differentiate self and non-self (Nancy et al., 2012), a mechanism that may be perturbed by the presence of MPs. In fact, it is reported that, once present in the human body, MPs may accumulate and exert localized toxicity by inducing and/or enhancing immune responses and, hence, potentially reducing the defence mechanisms against pathogens and altering the utilization of energy stores (Wright and Kelly, 2017).

Microplastics may access the bloodstream and reach placenta from the maternal respiratory system (Schlesinger, 1988) and the gastrointestinal tract (GIT) (Arumugasaamy et al., 2019), by means of M cells-





(caption on next page)

Fig. 3. A-B-C-D. Hypothetical mechanisms by which microplastics penetrate human tissues. (A) Endocytosis by M cells. At the level of the Peyer's Patched, below the mucous gut, MPs ingested with food can be uptaken by endocytosis from the M cells, transported across the epithelium into the subepithelial dome where they encounter dendritic cells, which in turn transport them through the lymphatic circulation, from where they reach the blood. (B) Paracellular Diffusion. MPs could penetrate through the intestinal lumen from loose junctions. This phenomenon could partially explain why some inflammatory states, which increase loose junctions favour intestinal passage. Once the intestinal lumen has been crossed, the MPs are collected by the dendritic cells and transported in the lymphatic and subsequently in the systemic circulation. (C) Upper airways, At the level of the upper respiratory tract the mucus is thicker and allows a successful clearance of the foreign bodies particles, in addition, the mechanical movement of ciliated epithelium and the presence of surfactant prevents smaller particles from spreading through the epithelium and reach the circulation. (D) Lower airways, In the lower respiratory tract the mucus layer is thinner, thus facilitating the diffusion of particles which, thanks to their particular aerodynamic shape, are able to reach this part of the respiratory tract. Once penetrated, the MPs can spread into the general circulation by cellular uptake or diffusion. (*Modified from: Mowat, A. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol* ***3,*** *331–341 (2003). https://doi.org/10.1038/nri1057. And Ruge, C. A.; Kirch, J.; Lehr, C. M. Pulmonary drug delivery: From generating aerosols to overcoming biological barriers-therapeutic possibilities and technological challenges. Lancet. Respir. Med. 2013, 1(5), 402–413.)*

mediated endocytosis mechanisms or paracellular transport. The most probable transport route for MPs is a mechanism of particle uptake and translocation, already described for the internalization from the GIT (Smith et al., 2018). The subsequent translocation to secondary target organs, usually associated with inflammatory responses in the surrounding tissues, such as the immune activation of macrophages and the production of cytokines (Hicks et al., 1996), depends on several factors, including hydrophobicity, surface charge, surface functionalization and the associated protein corona, and particle size.

Once MPs have reached the maternal surface of the placenta, as other exogenous materials, they can invade the tissue in depth by several transport mechanisms, both active and passive, that are not clearly understood yet (Tetro et al., 2018). The transplacental passage of 5–10 μm size MPs may depend on different physiological conditions and genetic characteristics. This might explain, together with the diverse food habits and lifestyle of patients, the absence of MPs in 2 of the 6 analyzed placentas and the different localization and characteristics of the particles identified in the present study. It is known that a great variability exists in the expression and function of placental drug transporters, both within human populations (inter-individual variability) and also during gestation (intra-individual variability) (Staud and Ceckova, 2015). We suppose that this variability exists also in relation to the mechanism of particles' internalization.

Potentially, MPs, and in general microparticles, may alter several cellular regulating pathways in placenta, such as immunity mechanisms during pregnancy, growth-factor signalling during and after implantation, functions of atypical chemokine receptors governing maternal-foetal communication, signalling between the embryo and the uterus, and trafficking of uterine dendritic cells, natural killer cells, T cells and macrophages during normal pregnancy. All these effects may lead to adverse pregnancy outcomes including preeclampsia and fetal growth restriction (Ilekis et al., 2016).

In conclusion, this study sheds new light on the level of human exposure to MPs and microparticles in general. Due to the crucial role of placenta in supporting the foetus development and in acting as an interface between the latter and the external environment, the presence of exogenous and potentially harmful (plastic) particles is a matter of great concern. Possible consequences on pregnancy outcomes and foetus are the transgenerational effects of plasticizer on metabolism and reproduction (Lee, 2018). Further studies need to be performed to assess if the presence of MPs in human placenta may trigger immune responses or may lead to the release of toxic contaminants, resulting harmful for pregnancy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aguayo, T., Clavijo, E., Villagràn, A., Espinosa, F., Sagues, F.E., Campos-Vallette, M., 2010. Raman vibrational study of pigments with patrimonial interest for the Chilean cultural heritage. J. Chil. Chem. Soc. 55, 347–351. https://doi.org/10.4067/S0717-97072010000300016.

Andreassen, E., 1999. Infrared and Raman spectroscopy of polypropylene. In: Karger-Kocsis, J. (Ed.), Polypropylene: An A-Z Reference. Kluwer Publishers, Dordrecht, Dordrecht. https://doi.org/10.1007/978-94-011-4421-6.

Arumugasaamy, N., Navarro, J., Kent Leach, J., Kim, P.C.W., Fisher, J.P., 2019. In vitro models for studying transport across epithelial tissue barriers. Ann. Biomed. Eng. 47, 1–21. https://doi.org/10.1007/s10439-018-02124-w.

Barboza, L.G.A., Dick Vethaak, A., Lavorante, B.R.B.O., Lundebye, A.-K., Guilhermino, L., 2018. Marine microplastic debris: An emerging issue for food security, food safety and human health. Mar. Pollut. Bull. 133, 336–348. https://doi.org/10.1016/j.marpolbul.2018.05.047.

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Glob. Chang. Biol. 24, 1405–1416. https://doi.org/10.1111/gcb.14020.

Dehaut, A., Cassone, A.-L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233. https://doi.org/10.1016/j.envpol.2016.05.018.

Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci. Rep. 7, 46687. https://doi.org/10.1038/srep46687.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA J. 14, 4501–4531. https://doi.org/10.2903/j.efsa.2016.4501.

European Chemical Agency [WWW Document], n.d. URL https://echa.europa.eu.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A. E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N. P., Lusher, A.L., Wagner, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ. Sci. Technol. 53, 1039–1047. https://doi.org/10.1021/acs.est.8b05297.

D.G. Hicks A.R. Judkins J.Z. Sickel R.N. Rosier J.E. Puzas R.J. O'Keefe Granular histiocytosis of pelvic lymph nodes following total hip arthroplasty. The presence of wear debris, cytokine production, and immunologically activated macrophages* J. Bone Jt. Surg. 78 1996 482 96 10.2106/00004623-199604000-00002.

Ilekis, J.V., Tsilou, E., Fisher, S., Abrahams, V.M., Soares, M.J., Cross, J.C., Zamudio, S., Illsley, N.P., Myatt, L., Colvis, C., Costantine, M.M., Haas, D.M., Sadovsky, Y., Weiner, C., Rytting, E., Bidwell, G., 2016. Placental origins of adverse pregnancy outcomes: potential molecular targets: an Executive Workshop Summary of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Am. J. Obstet. Gynecol. 215, S1–S46. https://doi.org/10.1016/j.ajog.2016.03.001.

Imhof, H.K., Laforsch, C., Wiesheu, A.C., Schmid, J., Anger, P.M., Niessner, R., Ivleva, N. P., 2016. Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes. Water Res. 98, 64–74. https://doi.org/10.1016/j.watres.2016.03.015.

Käppler, A., Fischer, D., Oberbeckmann, S., Schernewski, G., Labrenz, M., Eichhorn, K.-J., Voit, B., 2016. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? Anal. Bioanal. Chem. 408, 8377–8391. https://doi.org/10.1007/s00216-016-9956-3.

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y. Bin, Salamatinia, B., 2017a. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485–494. https://doi.org/10.1016/j.scitotenv.2016.10.213.

Karami, A., Golieskardi, A., Keong Choo, C., Larat, V., Galloway, T.S., Salamatinia, B., 2017b. The presence of microplastics in commercial salts from different countries. Sci. Rep. 7, 46173. https://doi.org/10.1038/srep46173.

Kosuth, M., Mason, S.A., Wattenberg, E.V., 2018. Anthropogenic contamination of tap water, beer, and sea salt. PLoS One 13, e0194970. https://doi.org/10.1371/journal.pone.0194970.

Lee, D.-H., 2018. Evidence of the possible harm of endocrine-disrupting chemicals in humans: ongoing debates and key issues. Endocrinol. Metab. 33, 44. https://doi.org/10.3803/EnM.2018.33.1.44.

Nancy, P., Tagliani, E., Tay, C.-S., Asp, P., Levy, D.E., Erlebacher, A., 2012. Chemokine gene silencing in decidual stromal cells limits T Cell access to the maternal-fetal interface. Science (80-.) 336, 1317–1321. https://doi.org/10.1126/science.1220030.

Notarstefano, V., Gioacchini, G., Byrne, H.J., Zacà, C., Sereni, E., Vaccari, L., Borini, A., Carnevali, O., Giorgini, E., 2019. Vibrational characterization of granulosa cells from patients affected by unilateral ovarian endometriosis: New insights from infrared and Raman microspectroscopy. Spectrochim. Acta Part A Mol Biomol. Spectrosc. 212, 206–214. https://doi.org/10.1016/j.saa.2018.12.054.

Notarstefano, V., Sabbatini, S., Pro, C., Belloni, A., Orilisi, G., Rubini, C., Byrne, H.J., Vaccari, L., Giorgini, E., 2020. Exploiting Fourier Transform InfraRed and Raman microspectroscopies on Cancer Stem Cells from Oral Squamous Cells Carcinoma: new evidence of acquired cisplatin chemoresistance. Analyst. https://doi.org/10.1039/D0AN01623C.

PlasticsEurope, 2016. Plastics – the Facts 2016 An analysis of European plastics production, demand and waste data [WWW Document]. URL https://www.plasticseurope.org/application/files/4315/1310/4805/plastic-the-fact-2016.pdf.

PrabhuDas, M., Bonney, E., Caron, K., Dey, S., Erlebacher, A., Fazleabas, A., Fisher, S., Golos, T., Matzuk, M., McCune, J.M., Mor, G., Schulz, L., Soares, M., Spencer, T., Strominger, J., Way, S.S., Yoshinaga, K., 2015. Immune mechanisms at the maternal-fetal interface: perspectives and challenges. Nat. Immunol. 16, 328–334. https://doi.org/10.1038/ni.3131.

Reineke, J.J., Cho, D.Y., Dingle, Y.-T., Morello, A.P., Jacob, J., Thanos, C.G., Mathiowitz, E., 2013. Unique insights into the intestinal absorption, transit, and subsequent biodistribution of polymer-derived microspheres. Proc. Natl. Acad. Sci. 110, 13803–13808. https://doi.org/10.1073/pnas.1305882110.

Ribeiro-Claro, P., Nolasco, M.M., Araújo, C., 2017. Characterization of microplastics by Raman spectroscopy. Compr. Anal. Chem. 75, 119–151. https://doi.org/10.1016/bs.coac.2016.10.001.

Scherrer, N.C., Stefan, Z., Francoise, D., Annette, F., Renate, K., 2009. Synthetic organic pigments of the 20th and 21st century relevant to artist's paints: Raman spectra reference collection. Spectrochim Acta Part A Mol. Biomol. Spectrosc. 73, 505–524. https://doi.org/10.1016/j.saa.2008.11.029.

Schlesinger, R.B., 1988. Biological disposition of airborne particles: basic principles and application to vehicular emissions. In: Watson, A.Y., Bates, R.R., Kennedy, D. (Eds.), Air Pollution, the Automobile, and Public Health.

Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T., Liebmann, B., 2019. Detection of various microplastics in human stool. Ann. Intern. Med. 171, 453. https://doi.org/10.7326/M19-0618.

Schymanski, D., Goldbeck, C., Humpf, H.-U., Fürst, P., 2018. Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water. Water Res. 129, 154–162. https://doi.org/10.1016/j.watres.2017.11.011.

Sklute, E.C., Kashyap, S., Dyar, M.D., Holden, J.F., Tague, T., Wang, P., Jaret, S.J., 2018. Spectral and morphological characteristics of synthetic nanophase iron (oxyhydr) oxides. Phys. Chem. Miner. 45, 1–26. https://doi.org/10.1007/s00269-017-0897-y.

SLOPP Library of Microplastics [WWW Document], n.d. URL https://rochmanlab.com/slopp-and-slopp-e-raman-spectral-libraries-for-microplastics-research.

Smith, D.J., Leal, L.G., Mitragotri, S., Shell, M.S., 2018. Nanoparticle transport across model cellular membranes: when do solubility-diffusion models break down? J. Phys. D. Appl. Phys. 51, 294004. https://doi.org/10.1088/1361-6463/aacac9.

Socrates, G., 2001. Infrared and Raman Characteristic Group Frequencies. Tables and chartsGeorge Socrates John Wiley and Sons, Ltd, Chichester, third ed., 2001. Price £135. John Wiley & Sons, Ltd. https://doi.org/10.1002/jrs.1238.

Staud, F., Ceckova, M., 2015. Regulation of drug transporter expression and function in the placenta. Expert Opin. Drug Metab. Toxicol. 11, 533–555. https://doi.org/10.1517/17425255.2015.1005073.

Stoye, D., Freitag, W. (Eds.), 1998. Paints, Coatings and Solvents. Wiley. https://doi.org/10.1002/9783527611867.

Testa-Anta, M., Ramos-Docampo, M.A., Comesaña-Hermo, M., Rivas-Murias, B., Salgueiriño, V., 2019. Raman spectroscopy to unravel the magnetic properties of iron oxide nanocrystals for bio-related applications. Nanoscale Adv. 1, 2086–2103. https://doi.org/10.1039/C9NA00064J.

Tetro, N., Moushaev, S., Rubinchik-Stern, M., Eyal, S., 2018. The placental barrier: the gate and the fate in drug distribution. Pharm. Res. 35, 71. https://doi.org/10.1007/s11095-017-2286-0.

Vaseashta, A. (Ed.), 2015. Life Cycle Analysis of Nanoparticles: risk, assessment, and sustainability. DEStech Publications, Inc.

Wright, S.L., Kelly, F.J., 2017. Plastic and Human Health: A Micro Issue? Environ. Sci. Technol. 51, 6634–6647. https://doi.org/10.1021/acs.est.7b00423.

WILL PARSON/CHESAPEAKE BAY PROGRAM

Microplastics collected from the Magothy River in Maryland.

MICROPLASTICS ARE EVERYWHERE — BUT ARE THEY HARMFUL?

Scientists are rushing to study the tiny plastic specks that are in marine animals — and in us.

By XiaoZhi Lim

Dunzhu Li used to microwave his lunch each day in a plastic container. But Li, an environmental engineer, stopped when he and his colleagues made a disturbing discovery: plastic food containers shed huge numbers of tiny specks — called microplastics — into hot water. "We were shocked," Li says. Kettles and baby bottles also shed microplastics, Li and other researchers, at Trinity College Dublin, reported last October[1]. If parents prepare baby formula by shaking it up in hot water inside a plastic bottle, their infant might end up swallowing more than one million microplastic particles each day, the team calculated.

What Li and other researchers don't yet know is whether this is dangerous. Everyone eats and inhales sand and dust, and it's not clear if an extra diet of plastic specks will harm us. "Most of what you ingest is going to pass straight through your gut and out the other end," says Tamara Galloway, an ecotoxicologist at the University of Exeter, UK. "I think it is fair to say the potential risk might be high," says Li, choosing his words carefully.

Researchers have been worried about the potential harms of microplastics for almost 20 years — although most studies have focused on the risks to marine life. Richard Thompson, a marine ecologist at the University of Plymouth, UK, coined the term in 2004 to describe plastic particles smaller than 5 millimetres across, after his team found them on British beaches. Scientists have since seen microplastics everywhere they have looked: in deep oceans; in Arctic snow and Antarctic ice; in shellfish, table salt, drinking water and beer; and drifting in the air or falling with rain over mountains and cities. These tiny pieces could take decades or more to degrade fully. "It's almost certain that there is a level of exposure in just about all species," says Galloway.

The earliest investigations of microplastics focused on microbeads found in personal-care products, and pellets of virgin plastic that can escape before they are moulded into objects, as well as on fragments that slowly erode from discarded bottles and other large debris. All these wash into rivers and oceans: in 2015, oceanographers estimated there were between 15 trillion and 51 trillion microplastic particles floating in surface waters worldwide. Other sources of microplastic have since been identified: plastic specks shear off from car tyres on roads and synthetic microfibres shed

from clothing, for instance. The particles blow around between sea and land, so people might be inhaling or eating plastic from any source.

From limited surveys of microplastics in the air, water, salt and seafood, children and adults might ingest anywhere from dozens to more than 100,000 microplastic specks each day, Albert Koelmans, an environmental scientist at Wageningen University in the Netherlands, reported this March[2]. He and his colleagues think that in the worst cases, people might be ingesting around the mass of a credit card's worth of microplastic a year.

Regulators are taking the first step towards quantifying the risk to people's health – measuring exposure. This July, the California State Water Resources Control Board, a branch of the state's environmental protection agency, will become the world's first regulatory authority to announce standard methods for quantifying microplastic concentrations in drinking water, with the aim of monitoring water over the next four years and publicly reporting the results.

Evaluating the effects of tiny specks of plastic on people or animals is the other half of the puzzle. This is easier said than done. More than 100 laboratory studies have exposed animals, mostly aquatic organisms, to microplastics. But their findings – that exposure might lead some organisms to reproduce less effectively or suffer physical damage – are hard to interpret because microplastics span many shapes, sizes and chemical compositions, and many of the studies used materials that were quite unlike those found in the environment.

The tiniest specks, called nanoplastics – smaller than 1 micrometre – worry researchers most of all (see 'Microplastics to scale'). Some might be able to enter cells, potentially disrupting cellular activity. But most of these particles are too small for scientists even to see; they were not counted in Koelmans' diet estimates, for instance, and California will not try to monitor them.

One thing is clear: the problem will only grow. Almost 400 million tonnes of plastics are produced each year, a mass projected to more than double by 2050. Even if all plastic production were magically stopped tomorrow, existing plastics in landfills and the environment – a mass estimated at around 5 billion tonnes – would continue degrading into tiny fragments that are impossible to collect or clean up, constantly raising microplastic levels. Koelmans calls this a "plastic time bomb".

"If you ask me about risks, I am not that frightened today," he says. "But I am a bit concerned about the future if we do nothing."

Modes of harm

Researchers have several theories about how plastic specks might be harmful. If they're small enough to enter cells or tissues, they might irritate just by being a foreign presence – as with the long, thin fibres of asbestos, which can inflame lung tissue and lead to cancer. There's a potential parallel with air pollution: sooty specks from power plants, vehicle exhausts and forest fires called PM_{10} and $PM_{2.5}$ – particulate matter measuring 10 µm and 2.5 µm across – are known to deposit in the airways and lungs, and high concentrations can damage respiratory systems. Still, PM_{10} levels are thousands of times higher than the concentrations at which microplastics have been found in air, Koelmans notes.

> **"It's almost certain that there is a level of exposure in just about all species."**

The larger microplastics are more likely to exert negative effects, if any, through chemical toxicity. Manufacturers add compounds such as plasticizers, stabilizers and pigments to plastics, and many of these substances are hazardous – for example, interfering with endocrine (hormonal) systems. But whether ingesting microplastics significantly raises our exposure to these chemicals depends on how quickly they move out of the plastic specks and how fast the specks travel through our bodies – factors that researchers are only beginning to study.

Another idea is that microplastics in the environment might attract chemical pollutants and then deliver them into animals that eat the contaminated specks. But animals ingest pollutants from food and water anyway, and it's even possible that plastic specks, if largely uncontaminated when swallowed, could help to remove pollutants from animal guts. Researchers still can't agree on whether pollutant-carrying microplastics are a significant problem, says Jennifer Lynch, a marine biologist affiliated with the US National Institute of Standards and Technology in Gaithersburg, Maryland.

Perhaps the simplest mode of harm – when it comes to marine organisms, at least – might be that organisms swallow plastic specks of no nutritional value, and don't eat enough food to survive. Lynch, who also leads the Center for Marine Debris Research at Hawaii Pacific University in Honolulu, has autopsied sea turtles that are found dead on beaches, looking at plastics in their guts and chemicals in their tissues. In 2020, her team completed a set of analyses for 9 hawksbill turtle hatchlings, under 3 weeks old. One hatchling, only 9 centimetres long, had 42 pieces of plastic in its gastrointestinal tract. Most were microplastics.

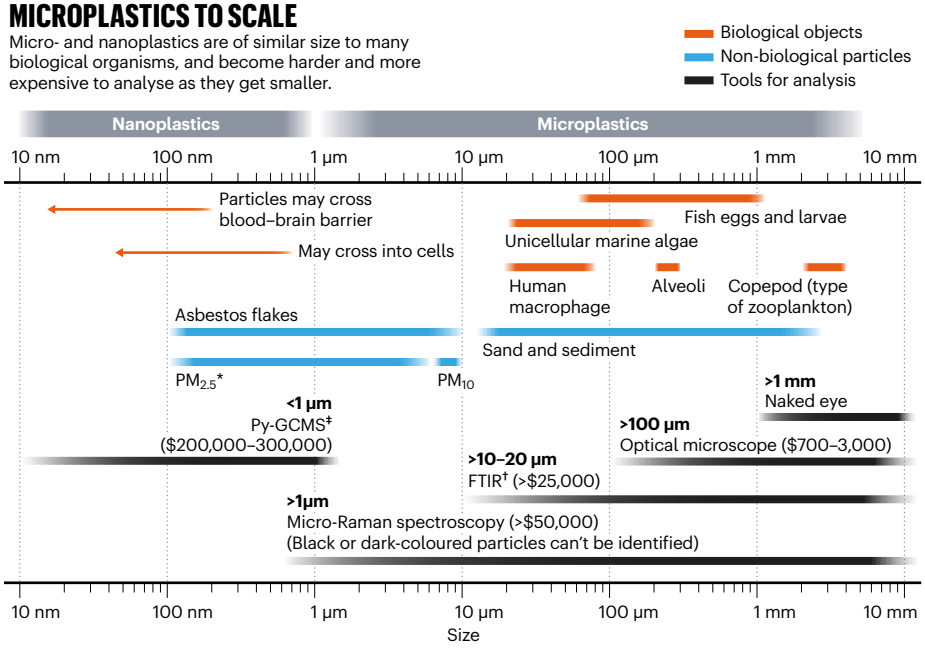
"We don't believe any of them died specifically from plastics," Lynch says. But she wonders whether the hatchlings might have struggled to grow as fast as they need to. "It's a very tough stage of life for those little guys."

Marine studies

Researchers have done the most work on microplastic risks to marine organisms. Zooplankton, for instance, among the smallest marine organisms, grow more slowly and reproduce less successfully in the presence of microplastics, says Penelope Lindeque, a marine biologist at the Plymouth Marine Laboratory, UK: the animals' eggs are smaller and less likely to hatch. Her experiments show

MICROPLASTICS TO SCALE

Micro- and nanoplastics are of similar size to many biological organisms, and become harder and more expensive to analyse as they get smaller.

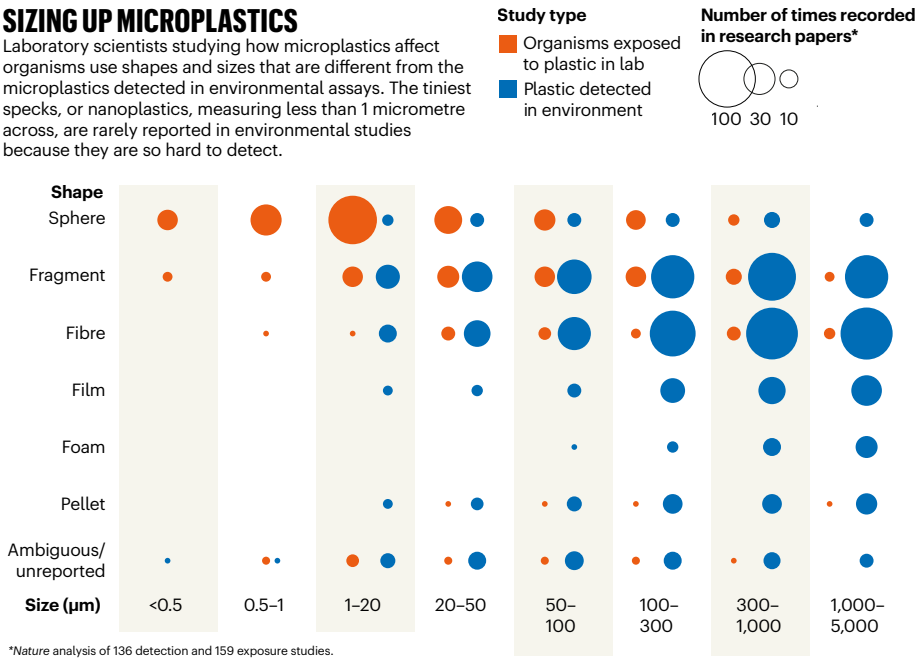


*Particulate matter less than 2.5 micrometres ($PM_{2.5}$) or less than 10 µm (PM_{10}) in diameter, often from soot, vehicle exhaust or dust; †FTIR, Fourier-transform infrared spectroscopy; ‡Py-GCMS, pyrolysis–gas chromatography–mass spectrometry.

SOURCE (TOOLS AND COSTS): S. PRIMPKE *ET AL. APPL. SPECTROSC.* **74**, 1012–1047 (2020).

SIZING UP MICROPLASTICS

Laboratory scientists studying how microplastics affect organisms use shapes and sizes that are different from the microplastics detected in environmental assays. The tiniest specks, or nanoplastics, measuring less than 1 micrometre across, are rarely reported in environmental studies because they are so hard to detect.



*Nature analysis of 136 detection and 159 exposure studies.

SOURCE: *NATURE* ANALYSIS

that the reproduction problems stem from the zooplankton not eating enough food[3].

But, because ecotoxicologists started running experiments before they knew what kinds of microplastics exist in aquatic environments, they depended heavily on manufactured materials, typically using polystyrene spheres of smaller sizes and at concentrations much higher than surveys found (see 'Sizing up microplastics').

Scientists have started shifting to more environmentally realistic conditions and using fibres or fragments of plastics, rather than spheres. Some have started coating their test materials in chemicals that mimic biofilms, which appear to make animals more likely to eat microplastics.

Fibres seem to be a particular problem. Compared with spheres, fibres take longer to pass through zooplankton, Lindeque says. In 2017, Australian researchers reported that zooplankton exposed to microplastic fibres produced half the usual number of larvae and that the resulting adults were smaller. The fibres were not ingested, but the researchers saw that they interfered with swimming, and identified deformations in the organisms' bodies[4]. Another study[5] in 2019 found that adult Pacific mole crabs (*Emerita analoga*) exposed to fibres lived shorter lives.

Most laboratory studies expose organisms to one type of microplastic, of a specific size, polymer and shape. In the natural environment, organisms are exposed to a mixture, says Koelmans. In 2019, he and his doctoral student Merel Kooi plotted the abundances of microplastics reported from 11 surveys of oceans, rivers and sediment, to build models of mixtures in aquatic environments.

Last year, the two teamed up with colleagues to use this model in computer simulations that predict how often fish would encounter microplastics small enough to eat, and the likelihood of eating enough specks to affect growth. The researchers found that at current microplastic pollution levels, fish run that risk at 1.5% of locations checked for microplastics[6]. But there are likely to be hotspots where the risks would be higher, says Koelmans. One possibility is the deep sea: once there, and often buried in sediment, it is unlikely the microplastics will travel elsewhere and there is no way to clean them up.

The oceans already face many stressors, which makes Lindeque more afraid that microplastics will further deplete zooplankton populations than that they will transfer up the food chain to reach people. "If we knock out something like zooplankton, the base of our marine food web, we'd be more worried about impacts on fish stocks and the ability to feed the world's population."

Human studies

No published study has yet directly examined the effects of plastic specks on people, leading researchers say. The only available studies rely on laboratory experiments that expose cells or human tissues to microplastics, or use animals such as mice or rats. In one study[7], for instance, mice fed large quantities of microplastics showed inflammation in their small intestines. Mice exposed to microplastics in two studies had a lowered sperm count[8] and fewer, smaller pups[9], compared with control groups. Some of the *in vitro* studies on human cells or tissues also suggest toxicity. But, just as with the marine studies, it's not clear that the concentrations used are relevant to what mice — or people — are exposed to. Most of the studies also used polystyrene spheres, which don't represent the diversity of microplastics that people ingest. Koelmans also points out that these studies are among the first of their kind, and could end up being outliers once there's an established body of evidence. There are more *in vitro* studies than animal studies, but researchers say they still don't know how to extrapolate the effects of solid plastic specks on tissues to possible health problems in whole animals.

One question surrounding risk is whether microplastics could remain in the human body, potentially accumulating in some tissues. Studies in mice have found that microplastics around 5 µm across could stay in the intestines or reach the liver. Using very limited data on how quickly mice excrete microplastics and the assumption that only a fraction of particles 1–10 µm in size would be absorbed into the body through the gut, Koelmans and colleagues estimate that a person might accumulate several thousand microplastic particles in their body over their lifetime[2].

Some researchers have started to explore whether microplastics can be found in human tissue. In December, a team documented this for the first time in a study that looked at six placentas[10]. Researchers broke down the tissue with a chemical, then examined what was left, and ended up with 12 particles of microplastic in 4 of those placentas. Yet it's not impossible that these specks were the result of contamination when the placentas were collected or analysed, says Rolf Halden, an environmental-health engineer at Arizona State University in Tempe — although he commends the researchers for their efforts to avoid contamination, which included keeping delivery wards free of plastic objects, and for showing that a control set of blank materials taken through the same sample analysis was not contaminated. "There is a continuing challenge of demonstrating conclusively that a given particle actually originated in a tissue," he says.

Those who are worried by their microplastic exposure can reduce it, says Li. His work on kitchenware found that the amounts of plastic shed depend highly on temperature — which is why he's stopped microwaving food in plastic containers. To reduce issues with baby bottles, his team suggests that parents could rinse sterilized bottles with cool water that has been boiled in non-plastic kettles, so as to wash away any microplastics released during sterilization. And they can prepare baby formula in glass containers, filling feeding bottles after the milk has cooled. The team is now recruiting parents to volunteer samples of their babies' urine and stools for microplastic analysis.

The nano fraction

Particles that are small enough to penetrate and hang around in tissues, or even cells, are the most worrying kind, and warrant more attention in environmental sampling, says Halden. One study[11] that deliberately let

pregnant mice inhale extremely tiny particles, for instance, later found the particles in almost every organ in their fetuses. "From a risk perspective, that's where the real concern is, and that's where we need more data."

To enter cells, particles generally need to be smaller than a few hundred nanometres. There was no formal definition of a nanoplastic until 2018, when French researchers proposed the upper size limit of 1 µm – tiny enough to remain dispersed through a water column where organisms can more easily consume them, instead of sinking or floating as larger microplastics do, says Alexandra ter Halle, an analytical chemist at Paul Sabatier University in Toulouse, France.

But researchers know almost nothing about nanoplastics; they are invisible and cannot simply be scooped up. Just measuring them has stumped scientists.

Researchers can use optical microscopes and spectrometers – which distinguish between particles by their differing interactions with light – to measure the length, width and chemical make-up of plastic particles down to a few micrometres. Below that scale, plastic particles become difficult to distinguish from non-plastic particles such as marine sediment or biological cells. "You're looking for the needle in the haystack, but the needle looks like the hay," says Roman Lehner, a nanomaterials scientist at the Sail and Explore Association, a Swiss non-profit research group.

In 2017, ter Halle and her colleagues proved for the first time that nanoplastic exists in an environmental sample: seawater collected from the Atlantic Ocean[12]. She extracted colloidal solids from the water, filtered away any particles larger than 1 µm, burnt what remained, and used a mass spectrometer – which fragments molecules and sorts the fragments by molecular weight – to confirm that plastic polymers had existed in the remnants.

That, however, gave no information on the exact sizes or shapes of the nanoplastics. Ter Halle got some idea by studying the surfaces of two degraded plastic containers she collected during the expedition. The top few hundred micrometres had become crystalline and brittle, she found; she thinks that this may also be true of the nanoplastics that probably broke off from these surfaces[13]. For now, because researchers cannot collect nanoplastics from the environment, those doing laboratory studies grind up their own plastic, expecting to get similar particles.

Using home-made nanoplastics has an advantage: researchers can introduce tags to help track the particles inside test organisms. Lehner and colleagues prepared fluorescent nano-sized plastic particles and placed them under tissue built from human intestinal-lining cells[14]. The cells did absorb the particles, but did not show signs of cytotoxicity.

> **"You're looking for the needle in the haystack, but the needle looks like the hay."**

Finding plastic specks lodged in intact slices of tissue – through a biopsy, for instance – and observing any pathological effects would be the final piece of the puzzle over microplastic risks, Lehner says. This would be "highly desirable", says Halden. But to reach tissues, the particles would have to be very small, so both researchers think it would be very difficult to detect them conclusively.

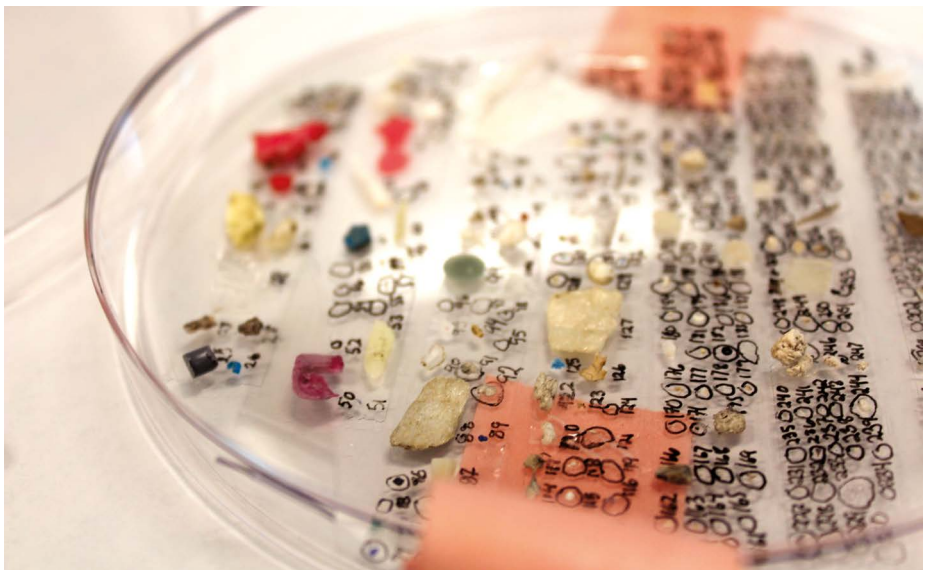
Collecting all these data will take a lot of time. Ter Halle has collaborated with ecologists to quantify microplastic ingestion in the wild. Analysing only particles larger than 700 µm in some 800 samples of insects and fish took thousands of hours, she said. The researchers are now examining the particles in the 25–700 µm range. "This is difficult and tedious, and this is going to take a long time to get the results," she says. To look at the smaller size range, she adds, "the effort is exponential."

No time to lose

For the moment, levels of microplastics and nanoplastics in the environment are too low to affect human health, researchers think. But their numbers will rise. Last September, researchers projected[15] that the amount of plastic added to existing waste each year – whether carefully disposed of in sealed landfills or strewn across land and sea – could more than double from 188 million tonnes in 2016 to 380 million tonnes in 2040. By then, around 10 million tonnes of this could be in the form of microplastics, the scientists estimated – a calculation that didn't include the particles continually being eroded from existing waste.

It is possible to rein in some of our plastic waste, says Winnie Lau at the Pew Charitable Trusts in Washington DC, who is the first author on the study. The researchers found that if every proven solution to curb plastic pollution were adopted in 2020 and scaled up as quickly as possible – including switching to systems of reuse, adopting alternative materials, and recycling plastic – the amount of plastic waste added could drop to 140 million tonnes per year by 2040.

By far the biggest gains would come from cutting out plastics that are used only once and discarded. "There's no point producing things that last for 500 years and then using them for 20 minutes," Galloway says. "It's a completely unsustainable way of being."



COLE BROOKSON

Microplastics collected in the San Francisco Bay area, labelled for study.

XiaoZhi Lim is a freelance writer in Medfield, Massachusetts.

1. Li, D. *et al. Nature Food* **1**, 746–754 (2020).
2. Nor, N. H. M., Kooi, M., Diepens, N. J. & Koelmans, A. A. *Environ. Sci. Technol.* **55**, 5084–5096 (2021).
3. Botterell, Z. L. R. *et al. Environ. Pollut.* **245**, 98–110 (2018).
4. Ziajahromi, S., Kumar A., Neale, P. A. & Leusch, F. D. L. *Environ. Sci. Technol.* **51**, 13397–13406 (2017).
5. Horn, D. A., Granek, E. F & Steele, C. L. *Limnol. Oceanogr. Lett.* **5**, 74–83 (2019).
6. Koelmans, A. A., Redondo-Hasselerharm, P. E., Nor, N. H. M. & Kooi, M. *Environ. Sci. Technol.* **54**, 12307–12315 (2020).
7. Li, B. *et al. Chemosphere* **244**, 125492 (2020).
8. Jin, H. *et al. J. Hazard. Mater.* **401**, 123430 (2021).
9. Park, E.-J. *et al. Toxicol. Lett.* **324**, 75–85 (2020).
10. Ragusa, A. *et al. Environ. Int.* **146**, 106274 (2021).
11. Fournier, S. B. *et al. Part. Fibre Toxicol.* **17**, 55 (2020).
12. Ter Halle, A. *et al. Environ. Sci. Technol.* **51**, 13689–13697 (2017).
13. Rowenczyk, L. *et al. Environ. Sci. Technol.* **54**, 4102–4109 (2020).
14. Caldwell, J. *et al. Environ. Sci. Nano* **8**, 502–513 (2021).
15. Lau, W. W. Y. *et al. Science* **369**, 1455–1461 (2020).

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Biodegradation of poly (vinyl alcohol) based materials

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Abstract

Poly(vinyl alcohol) (PVA) is recognized as one of the very few vinyl polymers soluble in water also susceptible of ultimate biodegradation in the presence of suitably acclimated microorganisms. Accordingly, increasing attention is devoted to the preparation of environmentally compatible PVA-based materials for a wide range of applications. The present article is aimed at providing a survey of the available information on the environmental fate of PVA and PVA-based materials. Literature data and recent advances on the biochemistry and microbial physiology of PVA biodegradation and on the influence of environmental conditions are discussed along with the biodegradation processes of other water-soluble materials. The biodegradation behaviors of several PVA-based materials including blends, composites and copolymers are also reviewed and discussed.

Keywords

Water-soluble; Poly(vinyl alcohol); Poly(vinyl acetate); Biodegradation; Blends; Composites

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Dominic Byrne[1], Geert Boeije[2], Ian Croft[3], Gerd Hüttmann[4], Gerard Luijkx[5], Frank Meier[6], Yash Parulekar[7] and Gerard Stijntjes[8]

Biodegradability of Polyvinyl Alcohol Based Film Used for Liquid Detergent Capsules

Questions and potential misperceptions have arisen about the potential contribution of liquid detergent capsules to the environmental microplastics issue. The film of these detergents is highly water soluble, also in cold water, as it must fully dissolve during every type of washing process. Water-soluble grades of polyvinyl alcohol, the most commonly used detergent capsule film material, are recognised to be biodegradable. In the current paper, adequate biodegradability is confirmed by means of ready biodegradation screening tests, across a range of polyvinyl alcohol detergent grade films. The high water solubility in itself implies that detergent capsule films are not within the microplastic scope. Furthermore, their biodegradability ensures there is no concern for persistence or accumulation in the environment.

Key words: Polyvinyl alcohol, Soluble film, Liquid detergent capsules, Biodegradation, OECD biodegradability screening test

Biologische Abbaubarkeit der für Flüssigwaschmittelkapseln verwendeten Folie auf Polyvinylalkoholbasis. Über den potenziellen Beitrag von Flüssigwaschmittelkapseln zur Mikroplastikproblematik in der Umwelt sind Fragen und mögliche Fehleinschätzungen aufgekommen. Die für Waschmittelkapseln verwendete Folie ist gut wasserlöslich, auch in kaltem Wasser, da sie sich in allen Waschprozessen vollständig auflösen muss. Wasserlösliche Polyvinylalkohole werden am häufigsten als Filmmaterial für Waschmittelkapseln verwendet und sind als biologisch abbaubar bekannt. In der vorliegenden Arbeit wird die ausreichende biologische Abbaubarkeit durch OECD Screening-Tests zur biologischen Abbaubarkeit für eine Reihe von Polyvinylalkohol-Waschmittelfolien bestätigt. Die gute Wasserlöslichkeit an sich impliziert, dass Waschmittelkapsel-Folien nicht in den Bereich des Mikroplastiks fallen. Darüber hinaus stellt ihre biologische Abbaubarkeit sicher, dass Bedenken hinsichtlich Persistenz oder Akkumulation in der Umwelt unbegründet sind.

Stichwörter: Polyvinylalkohol, Lösliche Folie, Flüssigwaschmittelkapseln, Biologischer Abbau, OECD-Test zur biologischen Abbaubarkeit

1 Introduction

1.1 Microplastic concerns

The unintentional release of microplastic into the aquatic environment (marine as well as freshwater) is of concern for human health and the ecosystem [1]. 'Microplastic' refers to microscopic solid particles (i.e. not water soluble) made of a synthetic polymer, with a high resistance to biodegradation. In the environment, progressive physical fragmentation into smaller and smaller particles can take place. Such particles are readily available for ingestion and are potentially liable to transfer within food chains. Further, microplastics are practically impossible to remove from the environment after release. These properties are known to result in exposure to a wide range of organisms including invertebrates, fish, marine reptiles, birds and cetaceans (either directly or via trophic transfer) and may also result in exposure to humans via food or water.

The definition of microplastic has been extensively assessed and discussed in Europe due to the proposed restriction of use of intentionally added microplastic in the European Economic Area. The European Commission initially defined microplastic as: 'synthetic water-insoluble polymers of 5 mm or less in any dimension' (EU Commission [2]). Subsequently, this definition was refined by the European Chemicals Agency: 'polymers that are (bio)degradable [are excluded]' (ECHA [1]); and latterly by ECHA's Committee for Risk Assessment: '[The term microplastic] means particles containing solid polymer, to which additives or other substances may have been added, and where ≥ 1 % w/w of particles have (i) all dimensions ≤ 5 mm, or (ii) a length of ≤ 15 mm and length to diameter ratio of >3… [this term] shall not apply to polymers that are (bio)degradable … with a water solubility > 2 g/l' (ECHA RAC [3]). According to this definition the water-soluble film used for liquid detergent capsules is not a microplastic. This is because the film size is outside of the microplastic range, and the film is both water-soluble and biodegradable – as further outlined in this paper.

Nevertheless, misperceptions do exist. For example, Thompson [4] incorrectly implies that polyvinyl alcohol (PVOH) from laundry detergent pods would be a common component in microplastic debris found in the environment. Thompson [4] sources its information from GESAMP [5], which in turn refers to Hidalgo-Ruz et al. [6] and to Vianello et al. [7]. The literature review by Hidalgo-Ruz et al. [6] indeed reports 3 earlier studies (out of a total of 42) in which

[1] A.I.S.E., Brussels, Belgium
[2] Boeije Consulting, Sint-Martens-Latem, Belgium
[3] McBride, Manchester, UK
[4] Reckitt Benckiser, Heidelberg, Germany
[5] Unilever, Rotterdam, The Netherlands
[6] Henkel, Düsseldorf, Germany
[7] Kuraray, Merrillville, USA
[8] Procter & Gamble, Strombeek-Bever, Belgium

PVOH was identified among sorted microplastic debris. However, the considered studies all predate the market entry of liquid laundry detergent capsules – which mainly occurred after 2010. Vianello et al. [7] sampled ten sites in the Venice Lagoon (Italy), and found PVOH in only one specific location, which was an industrial discharge. Claessens et al. [8] investigated microplastic in marine sediment near the Belgian coast. This study identified PVOH in fibres, and concluded that this was most likely originating from fishing lines. Microplastic films were also found, but all identified to be made of nylon.

In conclusion, the available literature does not indicate an association of PVOH film from detergent capsules with environmental microplastic. Nevertheless, it also shows that traces of PVOH from other sources can indeed be found in the marine environment. It is important to note that PVOH has many, very different applications. The overarching nomenclature of PVOH covers a broad variety of polymer designs – several of which do not exhibit the same water solubility and biodegradability as the PVOH grades used for detergent film applications. Thus, it is not surprising that such materials may indeed persist sufficiently long in the environment to be detectable.

Only a specific range of PVOH meets the performance requirements to be usable for detergent soluble film applications. Hence, it is crucial to have a sufficiently accurate and narrow identification of the PVOH polymer characteristics to assess the environmental fate profile of detergent film material. The objective of the current paper is to review the biodegradability of PVOH film that is used in detergent capsules, and to assess whether these materials may pose a risk for persistence in the environment. In addition, the paper aims to explore whether these films may be contributing to microplastic in the environment.

1.2 Detergent capsules

Detergent capsules are a fairly recent innovation in the laundry and home care sector. In this highly concentrated product form, all active ingredients are contained in a single unit dose capsule. This capsule dissolves after contact with water inside the washing machine or dishwasher, and then releases the detergent. This way, consumers can easily dose the correct amount of product without the need to measure, and without spills that may occur when dispensing a traditional free-flowing detergent product. In addition, the high level of concentration leads to lower product amounts.

The capsule material is made of a water-soluble film. This is generally based on PVOH, with polymer backbone modifications and specific performance additives. The films are developed such that they readily dissolve as intended in the washing process, including in cold water cycles. To avoid spillage and to ensure consumer safety, the soluble film is designed not to dissolve and rupture prematurely (e. g. when touched with wet hands, or when put into the mouth); to resist compression (e. g. when squeezed by a child); and to trigger an aversive reaction in case of oral contact. These features are required for liquid laundry detergent capsules in the EU under regulation (EU)1297/2014 (amending the CLP Regulation (EC)1272/2008), and are also included the voluntary ASTM Standard for this product category [9].

1.3 Polyvinyl alcohol

PVOH is a synthetic resin that is generally prepared by the saponification (hydrolysis) of polyvinyl acetate (Finch [10]). When all acetate groups have been converted to alcohol groups, 'fully hydrolysed' PVOH is obtained. On the other hand if a certain proportion of acetate groups are allowed to remain, the result is referred to as 'partially hydrolysed' PVOH (Figure 1). While in fact a vinyl alcohol – vinyl acetate copolymer, the latter is nevertheless also commonly referred to as PVOH.

PVOH has a wide variety of applications. It is a key ingredient in formulation processes in various industries including food packaging, construction, electronics, coatings, printing, textile, cosmetics, and paper. Demand is mainly driven by the food packaging industry, which accounted for 31.4% of the global volume in 2016 (Grand View Research [11]). PVOH is also extensively used in the construction industry as auxiliary component in cement-based composite materials (Thong et al. [12]). Other applications are in warp sizing and processing of textile fibres, in paper manufacturing (e. g. coating to make the paper more resilient to oils and grease), and in adhesives (Finch [10]). Muppalaneni and Omidian [13] provide an overview of medical and pharmaceutical applications, such as tablets and contact lenses, and surgical threads (Gaaz et al. [14]). PVOH is also used in sports fishing: PVOH bags filled with oil-based or dry fishing bait are attached to the hook, and as PVOH is soluble in water, when the bag lands on the bottom it leaves the hook bait surrounded by pellets and ground bait that attracts fish (American Carp Society [15]).

The wide diversity of applications of PVOH implies there is also a broad range of required physical-chemical characteristics, depending on the specific use. This is partially achieved by selecting an optimal polymer size and balance between vinyl acetate and vinyl alcohol in the copolymer. Further, it is achieved by means of modifications of the polymer backbone and by means of additives (e. g. plasticisers) that are blended into the resin. Different polymer modifications are described in Monosol [16], e. g. inclusion of an anionic monomer unit in the copolymer, or by having a carboxyl group modified copolymer. Consequently, when assessing PVOH used in detergent capsule applications, it is essential to focus on material with characteristics that are suitable for this specific application. Most importantly, as outlined above, PVOH used in a detergent film requires excellent solubility in cold water.

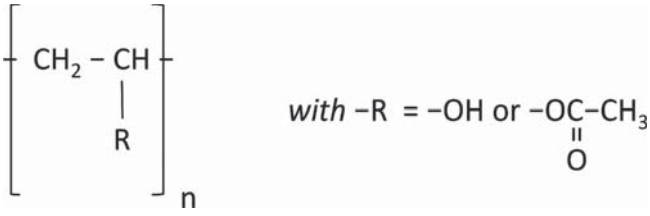

Figure 1 Chemical structure of PVOH (partially hydrolysed)

1.4 Water solubility of PVOH

The water solubility of PVOH depends mainly on its degree of hydrolysis (DH), and to a second extent on its degree of polymerization (DP) (Finch [10]). This is determined by two counteracting influences of the hydroxyl groups. On the one hand, the many hydroxyl groups cause PVOH to have a high affinity to water, which drives water solubility. On the other hand, the strong hydrogen bonding between inter- and intramolecular hydroxyl groups can greatly impede solubility in water. As a consequence, fully hydrolysed PVOH is highly crystalline and only dissolves in hot water (> 60 °C), whereas 'partially hydrolysed' PVOH is more weakly hydrogen-bonded, less crystalline, and generally soluble in cold water (< 10 °C) (Monosol [16]). Finch [10] shows high water solubility at ambient temperature (20 °C) for PVOH with a degree of hydrolysis up to 90 %, but rapidly dropping with further increasing DH. The observed negative effect of an increasing degree of polymerization (in the range of 500–2500) on solubility was of limited relevance for PVOH with a degree of hydrolysis < 90 %. This is illustrated in Figure 2 for a degree of polymerisation of 1750 (after Finch [10]). Note that for practical detergent applications, PVOH films are modified and contain additives, to ensure that they are 100 % soluble in cold water (as opposed to what is shown in Figure 2 for pure unmodified PVOH).

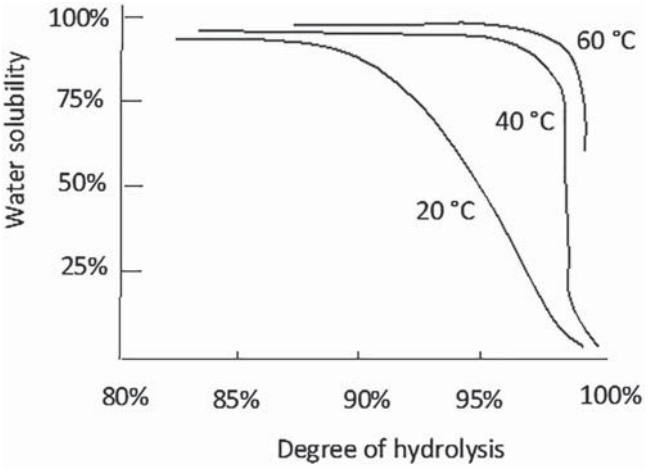

Figure 2 PVOH water solubility as a function of DH and temperature

Similarly, Amann and Minge [17] report that an optimum cold water solubility can be observed with a degree of hydrolysis of 87 %–89 % for a degree of polymerisation between 600 and 2 400, whereas a totally hydrolysed PVOH is almost insoluble in cold water and can only be dissolved by boiling in water for an extended period of time. Julinová et al. [18] confirm that the solubility of PVOH is highly reliant on the degree of hydrolysis: products with DH = 95 % are insoluble in cold water and only dissolve at 65 °C–70 °C; whereas when DH = 88 %, the PVOH is highly soluble in cold water. When DH drops below circa 80 %, the trend is reversed. With DH between 70 % and 80 %, precipitation of PVOH from the solution occurs at temperatures below 35 °C, and PVOH with a content of acetate groups more than 50 % becomes insoluble in water.

1.5 Biodegradability of PVOH

PVOH is recognised as one of the very few vinyl polymers soluble in water that is susceptible to ultimate biodegradation in the presence of suitably acclimated microorganisms. Chiellini et al. [19] provide a comprehensive overview of the biodegradability of PVOH. More recent reviews are presented in Kawai and Hu [20] and in Amann and Minge [17]. Chiellini et al. [19] conclude that the ultimate biological fate of PVOH depends largely upon the environment it reaches. High levels of biodegradation were observed in aqueous environments that contained acclimated bacterial species often associated with PVOH-contaminated waste water and sewage sludge. Similarly, Julinova et al. [18] state that in prior research, PVOH-degrading bacteria had been isolated from settings previously contaminated by PVOH, where adaptation could take place. However, from this, these authors deduce that in uncontaminated environments adapted bacteria may not be present, and conclude that PVOH is essentially non-biodegradable. They also mention that in many cases, PVOH passes through waste water treatment plants unchanged – although this is not substantiated, because the references provided describe research with a different material: poly(vinylpyrrolidone), not PVOH. Due to the broad down-the-drain emission of PVOH used in liquid detergent capsules (following wide dispersive consumer use), there is a steady load of PVOH into the domestic waste water pathway. As such, it is fair to assume that acclimation has taken place in sewage treatment activated sludge, or in the receiving environment in case of untreated emissions. Thus, the conclusions by Chiellini et al. [19] apply in this context.

Like a majority of polymers (e. g. polyolefins, polystyrenes and polyacrylates), PVOH has a carbon-carbon single bond backbone. However, unique among synthetic polymers, PVOH also exhibits a 1,3-diol structure that is quite common in natural materials such as carbohydrates. Unsurprisingly, as a general mechanism, the biodegradation of PVOH starts on the outside of the cells via enzymatic attack on these 1,3-diol repeating units. This results in a mixture of acetoxy-hydroxy and hydroxy fatty acids. Upon intracellular enzymatic de-acetylation, these can be further metabolised via the classical β-oxidation pathway and Krebs cycle. This mechanism is common to all PVOH-degrading microorganisms (Solaro et al. [21]). Among the degrading strains, many species can be found in the genus Pseudomonas and Sphingomonas (Yamamatsu et al. [22]). A simplified illustration of the proposed PVOH biodegradation pathway is given in Wilkes and Aristilde [23] (Figure 3).

In aqueous aerobic environments, biodegradation of PVOH runs parallel with its water solubility (Chiellini et al. [24]). Kawai and Hu [20] report that the biodegradation of PVOH is affected by chemical structural characteristics, such as stereotacticity and 1,2-diol units. It was not found to be significantly influenced by the DP when this was in the range of 10–2 000 (which implies the DP is not a driving factor for PVOH used in detergent capsule films). The effect of the DH is not entirely clear. DH was not found to have a substantial impact when above 80 % while below a level impeding solubility (several references quoted in Chiellini et al. [19]). Hatanaka et al. [25] saw a reduction of biodegradation activity as the DH lowered. Chiellini et al. [26] observed a

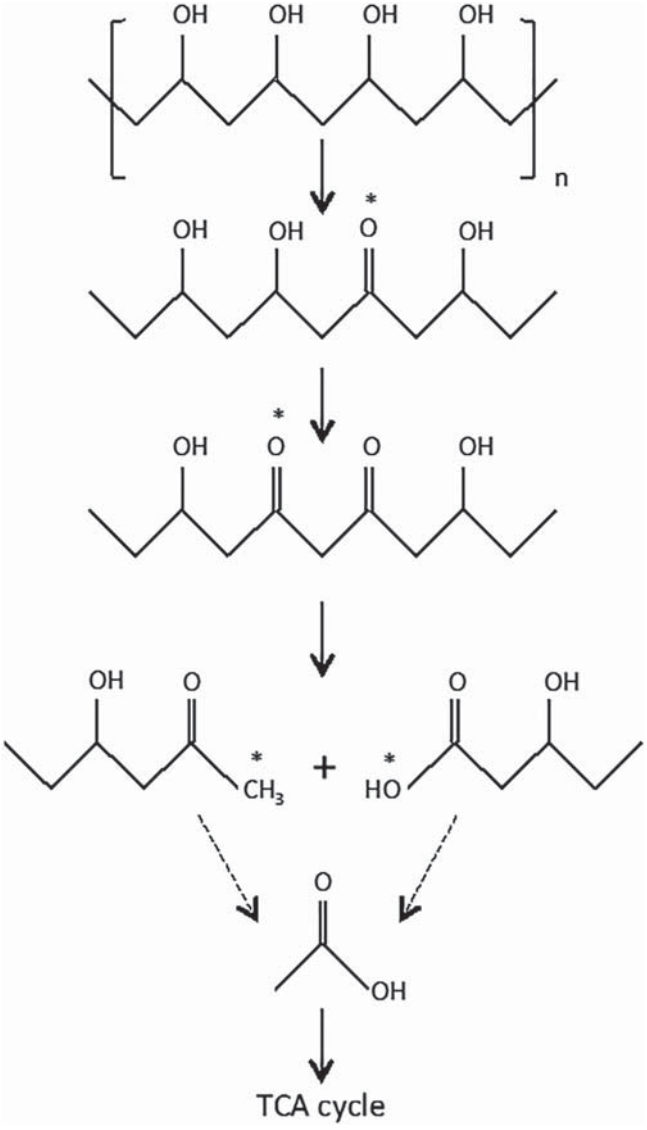

Figure 3 Proposed PVOH biodegradation pathway (after Wilkes and Aristilde [23])

similar extent of biodegradation with DH = 72%, 88% and 98%, but the lag phase with the DH = 72% material lasted 10 days longer than with the other materials.

Studies conducted with cultures or environmental inoculums that had not been previously exposed to PVOH tended to result in fairly slow degradation rates. On the other hand, for example in presence of sludge taken from a paper mill wastewater treatment system (well-adapted to PVOH), the degradation of PVOH proceeded at comparable rates as for cellulose (Pajak et al. [27]). Hence, adaptation of the microbial community was found to have a favourable impact on the biodegradation rate of PVOH. The effect of adaptation is also illustrated in the guidelines for the OECD 302B (Zahn-Wellens/EMPA test) [28]. In this guideline document, PVOH is used as an example of a substance that exhibits an adaptation phase followed by a degradation phase in the test. In this particular example (Figure 4), near-complete biodegradation was reached both with adapted and non-adapted sludge, but without prior adaptation the process took about twice as long. This illustrates well that also without prior acclimation to PVOH, microbial communities will eventually be able to degrade this material. In this context, it should be noted that for widely used consumer products, such as detergents, there is a fairly constant and ongoing emission (rather than isolated peak exposures). Consequently, the microbial communities in the receiving environment and waste water treatment plants are generally well-adapted to the ingredients.

Matsumura et al. [29] demonstrated biodegradability of PVOH under anaerobic conditions, using anaerobic river sediments and anaerobically treated activated sludge from a sewage treatment plant. Marušincová et al. [30] found that PVOH was biodegraded under denitrifying conditions with a microbial community that originated from a municipal wastewater treatment plant. The derived microbial consortium was capable of PVOH degradation under both denitrifying and aerobic conditions.

Chiellini et al. [31] investigated the rates and extents of absorption and desorption of PVOH on different solid substrates. PVOH with a higher degree of hydrolysis was more adsorbed, whereas an increase in molecular weight decreased adsorption. Biodegradation experiments carried out in liquid cultures of PVOH that were adsorbed on montmorillonite

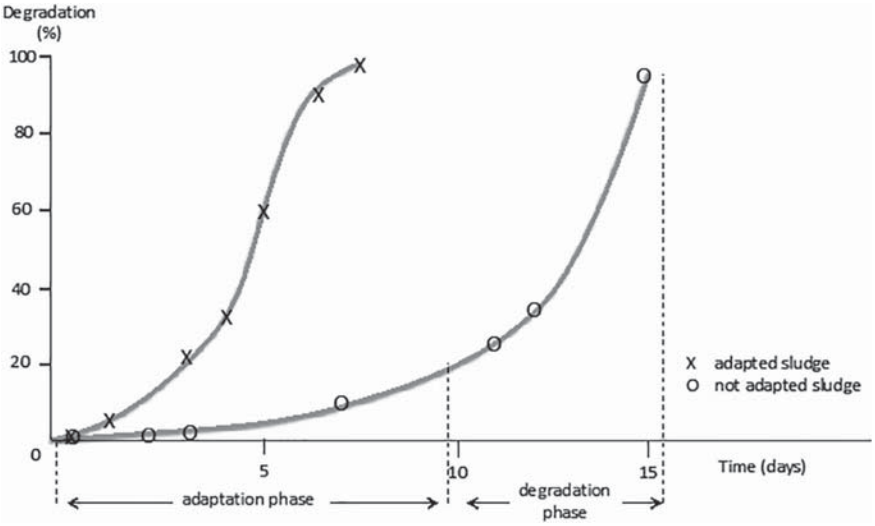

Figure 4 Example of sludge adaptation for PVOH in the OECD 302B test

clay showed that the mineralization of the adsorbed material was much lower than what had been detected for the non-adsorbed PVOH. Similarly, Chiellini et al. [26] had found lower rates and extents of PVOH mineralization in solid cultures in the presence of either soil or compost samples. Different hypotheses were suggested to account for these observations, such as the absence or scarce occurrence of PVOH-degrading microorganisms in soil and compost matrices, the physical state of the PVOH samples, and PVOH's strong interactions with the solid matrices (Chiellini et al. [19]).

Data about adsorption of detergent film grade PVOH to activated sludge are not available. QSAR modelling (EPI Suite, US EPA [32]) indicates that PVOH has a very low predicted hydrophobicity (e.g. calculated Log K_{ow} = –20 for DP = 50 and DH = 85%; further decreasing by approximately 5 for every increase of DP by 10 units) and consequently a very low tendency to adsorb to organic carbon, and to activated sludge, is predicted. Thus, the main fate pathway is expected to be aquatic/dissolved.

Meier et al. [33] report on a detergent case study, where the OECD 302 test was applied to assess the inherent biodegradability of a PVOH based film material. In this study, 85% of the dissolved organic carbon (DOC) was removed within 4 weeks. A parallel study demonstrated that 78% of the test material's carbon had been converted to CO_2 over a period of 2 months. These findings show that the PVOH film material can be qualified as 'inherently biodegradable' (cf. ECHA [34]). Consequently, it is not persistent and does not accumulate in aquatic environments. Nevertheless, ECHA [34] warns that positive inherent biodegradability test results in general should not be interpreted as conclusive evidence for rapid degradation in the environment.

For the environmental fate pathway of PVOH film used in detergent capsule applications, the relevant circumstances are the aquatic environment, under aerobic conditions. Indeed after use in the washing machine or dishwasher, the ingredients of detergent capsules – including the now dissolved PVOH – are discarded down the drain with the waste water. This is further conveyed via the sewer system, normally to a waste water purification facility, or worst case to an untreated discharge into surface water. Under all of these conditions, the literature shows that PVOH is biodegradable, and that especially the highly soluble variants of PVOH have the best biodegradation profile.

2 Experimental procedure

2.1 Data sources

Over the past decade, several companies (either detergent formulators or film producers) have commissioned ready biodegradation tests with PVOH film materials. For the purpose of the current analysis, data on 6 different films were available for review. These studies were conducted between 2010 and 2018. Because of confidentiality and competition law considerations, no information about the individual studies (neither on the test materials and their characteristics, nor on the individual results) can be publicly disclosed. Instead, the data were compiled confidentially by the European detergents industry association A.I.S.E., and were anonymised. In this paper an aggregation is provided of the different studies that were made available. Note that for materials where replicate test data had been shared, and/or materials that had been tested at more than one concentration, the average across replicates and test concentrations was used as a single value for the aggregation. The evolution of the biodegradation extent over time is presented as a cloud of all observation points for the different test materials, not identifying which are the actual materials tested, nor which data points belong together as part of one study.

All of the 6 tested film materials are confirmed to be (or to have been) used in the production of detergent capsules on the market. Thus, the DH and molecular weight (i.e. the DP) are representative of what is used in the market for detergent applications. These technical materials are not pure PVOH (i.e. a pure copolymer of vinyl alcohol and – a lower proportion of – vinyl acetate). First, the PVOH polymer backbone is typically modified to some extent, to address the unique needs of the detergent capsule application – e.g. cold water solubility with appropriate kinetics (sufficiently fast but not prematurely), adequate compression resistance, and processing ability. Second, additives are likely present, e.g. plasticisers, aversive agents to prevent oral exposure. These additives may have been blended into the resin or may have been coated on the film. Across the tested materials presented in this paper, the PVOH polymer resin percentage in the film material ranges from 60% to 85%. Consequently, the data presented herein reflect the holistic biodegradability profile of technical PVOH films (i.e. technical mixtures) as used for detergent capsule applications, rather than the biodegradability of pure PVOH in isolation.

2.2 Biodegradation studies

Different types of ready biodegradation test protocols were used across the available studies. These were all aerobic ready biodegradability screening tests. As a common principle, a solution of the test material is inoculated with a low amount of microorganisms, in the presence of mineral nutrients, but in absence of any other carbon source than the test material itself. These screening tests are intentionally conservative by design. Biodegradation will indeed only be observed in case the inoculated micro-organisms are able to source energy and carbon solely from the test material – and this in a sufficiently efficient way, to allow the exponential microbial growth that is required to establish a self-accelerating degradation of the material. As the microorganisms utilise the test material as a food source, part of it is taken up into the growing microbial biomass, and the remainder is converted into carbon dioxide, while in this process oxygen is consumed.

For 4 of the tested film materials, the OECD 301B approach was applied. In this test method, the amount of CO_2 produced over time (captured and quantified using a sodium hydroxide trap) is expressed as a percentage of the theoretical maximum, based on the total organic carbon analysis of the sample. The OECD 301F test was used for one material. This study monitors the oxygen uptake of the microorganisms instead of their CO_2 production. Finally, one material was tested according to OECD 310. Like under OECD 301B, CO_2 production is monitored as a measure of biodegradation, but in this case via an inorganic carbon analysis of the test vessels. The OECD 301 and 310 series of ready biodegradability tests are the standard for screening purposes. These tests are generally considered to be conser-

vative in nature. As such, ECHA considers a positive result in one of these tests to be unequivocal, not requiring further investigation of the biodegradability of the chemical or environmental effects of any transformation products [35].

Three OECD 301B studies were performed using 1 L treatments, dosed (in dissolved form) at 12 mg C/L. Freshly collected activated sludge from a primarily domestic wastewater treatment plant was used as inoculum and was added at a target concentration of 17 mg/L. An electrolytic respirometer test system was used for the study. A conductivity probe immersed in 0.75 % NaOH was used to measure the production of CO_2 and a pressure transducer monitored the decrease in pressure due to oxygen consumption. A sensor assembly charged with $CuSO_4$ continually equalised the pressure with oxygen as it was consumed.

A fourth OECD 301B study was conducted using a 3 L treatment volume, with the pre-dissolved test material dosed at 10 mg C/L, and the domestic activated sludge inoculum was added to 30 mg/L. Here the reactors were delivered CO_2-free air and were allowed to vent into a 0.20 % NaOH scrubber solution. Each scrubber solution was analysed for Total Inorganic Carbon concentrations periodically throughout the extent of the test to determine the amounts of CO_2 produced by each reactor.

In the OECD 301F test, the measurement of oxygen uptake was conducted in respirometer units with piezoresistive electronic pressure sensors in the bottle tops. Each individual unit consisted of a dark glass 500 mL bottle, with a magnetic stirrer. As inoculum, activated sludge from a predominantly domestic sewage treatment works was used, at a concentration of 30 mg/L in the final volume. 10 mg/L of dissolved test material was dosed in each unit, and the balance of the volume (total of 432 mL) was with standard mineral medium. Carbon dioxide produced by microbial respiration was absorbed by a KOH solution placed in a rubber sleeve in the neck of each bottle, and the oxygen taken up was measured as a decrease in pressure. The electronic controller collected the pressure values from the measuring tops and calculated the biological oxygen demand (as mg/L). Oxygen uptake was recorded automatically every 112 min during the experimental period. Oxygen uptake values were corrected for the inoculum blank, and the biodegradation was calculated as a percentage of the measured chemical oxygen demand for the test substance.

The OECD 310 test was carried out in 125 mL serum vessels, sealed with butyl rubber septa and aluminium crimp caps. The vessels were incubated in an incubator-shaker, shaking at 150 rpm. As inoculum, activated sludge from a predominantly domestic sewage treatment works was used, at a final sludge solids concentration of 4 mg/L. The test substance vessels contained pre-dissolved PVOH film at nominal 5.0 mg/L or 7.5 mg/L as carbon, in standard mineral medium. The total liquid volume in the test vessels was 100 mL. On sampling days, sacrificed vessels were made alkaline by the addition of a sodium hydroxide solution, to trap the produced CO_2. Samples taken from the liquid were then analysed for inorganic carbon. Mineralization of the test and reference materials was followed by measurement of carbon dioxide evolution on days 4, 7, and subsequent 7-day intervals. Biodegradation was calculated as the ratio of evolved inorganic carbon to the (measured) organic carbon added at the start of the study.

All of the above studies covered a 28-day test period. In addition, three studies were continued beyond this point, up to 60 days according to the REACH PBT enhanced ready biodegradation approach [35].

The above test protocols are biologically equivalent, despite differences in terms of execution and measured parameters. Importantly, the test material and inoculum concentrations are similar and in all methods the inoculum is from (predominantly) domestic activated sludge sewage treatment plants. None of the studies used pre-acclimated inoculum. Whether biodegradation activity is measured either via CO_2 production (OECD 301B) or via O_2 consumption (OECD 301F and OECD 310) is biologically inconsequential, because both are metrics (respectively output and input) of the same processes. As a reference value, in each of these methods a hypothetical maximum (100 %) is determined based on the assumed full decomposition of the test material. The pass level for biodegradability is the same for the three test methods, i. e. 60 % of the hypothetical maximum. Whereas the protocol differences may impact especially the start-up phase of the biodegradation studies (length of the lag phase, initial kinetics), the eventual outcome once the bacteria have achieved exponential growth is not expected to be fundamentally different. Consequently, for the purpose of the current assessment it was deemed appropriate to aggregate the results from OECD 301B, 301F and 310 studies into a single overview. To ensure confidentiality, only the first 28 days of all studies were included in the aggregation.

2.3 Interpretation

To help interpret the aggregated data set, mathematical modelling was applied. The logistic plot is generally used to estimate the rate constant for biodegradation and is applicable for sigmoidal degradation curves (Reuschenbach et al. [36]). The following equation (Larson et al. [37]) for the logistic model describes an S-shaped degradation curve in single-dose batch systems:

$$y = a \cdot \left(1 - e^{-k_1 \cdot t}\right)^{-1/n} \quad (1)$$

where y = cumulative percentage of biodegradation (%)
t = time (days)
a = asymptotic value for the cumulative percentage of biodegradation (%)
k_1 = first-order biodegradation rate constant (day^{-1})
n = empirical constant

This model was fitted to the data by means of numerical optimisation (Excel for Mac 2011, version 14.4.1, Solver GLG Non-linear engine). For each observed data point, the residual between the measured and modelled cumulative biodegradation percentages was calculated. Next, for each of the six different studies, the sum of the squared residuals across the data points in that study was determined. These sum of squares values for each individual study were then divided by the number of data points in that specific study – to ensure an equal weight for each of the studies (as without this last step, a higher weight would implicitly be given to studies with more data points). Finally, the overall sum of these metrics was calculated across the six studies. This overall sum was minimised by means of numerical optimisation of the three model parameters (a, k_1 and n).

3 Results and discussion

Each of the six materials in the data set exhibited a degradation profile that followed a sigmoidal pattern. The aggregated data (up to 28 days) are presented in Figure 5, accompanied by the fitted logistic model curve. For 3 of the tested materials, data were collected up to 60 days, however, this cannot be included in the chart to avoid confidentiality concerns (i.e. it shall not be possible to identify individual materials).

The extent of biodegradation after 28 days was 60.4 % on average across the six materials. Across the studies, the 28 days value ranged quite broadly, from 38 % up to 86 %. As outlined above, different study protocols had been used, that are fundamentally equivalent but that nevertheless could result in a different duration of the lag phase or different kinetics. As such, the relevant result to be compared across the studies is the eventual biodegradation percentage, rather than what happens during the first weeks of the study. For 2 out of the 6 materials, the 60 % threshold was not reached within 28 days. However, for these specific film materials, the enhanced OECD 301B protocol had been applied with measurements continuing up to 60 days. This showed a biodegradation extent well above the 60 % threshold in both cases, demonstrating that the result below 60 % on day 28 did not imply a lack of biodegradability.

The fitted logistic model has the following parameters: $a = 97.7\,\%$, $k_1 = 0.0567\ \text{day}^{-1}$, $n = -0.483$. The model calculation of the biodegradation extent at 28 days (60.8 %) closely matches the average of the observed data (60.4 %). The high asymptotic value a confirms the ongoing integral biodegradation, going well beyond the 60 % threshold over time.

All of the studies exhibited an initial lag phase, before the biodegradation process self-accelerated. The duration of the lag phase varied between the studies. This may potentially be explained by the fact that the test materials are complex mixtures, with varying auxiliary ingredients in addition to PVOH, and also by the fact that PVOH itself is a polydisperse material. But also differences in the adaptation to PVOH of the inoculum (domestic activated sludge) may have been a factor, especially in the older studies that were conducted at a time when detergent capsules did not yet have a broad market presence. The importance of adaptation for the biodegradation of PVOH has been well established in the literature [18, 19, 27, 28].

Note that the 10-day window concept is not relevant in the context of films because these are mixtures, not single substances. For perspective, the European Commission Scientific Committee on Toxicity, Ecotoxicity and the Environment deemed it not necessary to utilise the 10-day window criteria for assessing the ultimate biodegradability of surfactants in detergents – due to the different kinetics associated with different homologues present in many commercial surfactants [38]. Finally, the 10-day window is not relevant for a microplastics assessment, of which the main focus is absence of persistence, rather than the actual rate of biodegradation.

Extrapolating the fitted logistic model further in time (Figure 6) predicts the ultimate complete biodegradability of the PVOH films in this assessment.

The presented data confirm that the PVOH material used in detergent capsule film materials is biodegradable. As this was shown by means of the stringent OECD ready biodegradability screening tests, due to the conservative design of the studies, these positive results unequivocally indicate rapid and ultimate biodegradation in most environments (ECHA [35]). These findings are in line with what is reported in the literature on PVOH (e.g. [17, 19]) and on PVOH based detergent film [33].

The positive results in the screening biodegradation tests are not per se conflicting with literature that concluded poor biodegradability for PVOH [18], or that detected PVOH in environmental samples [5–8]. This is because a very broad range of applications for PVOH exist [11–15], each with specific requirements for the physical/chemical properties. These different properties are achieved via different polymer design strategies. Not all PVOH is equally water soluble, and neither is it equally biodegradable. Complete water solubility, also at low temperatures, is a prerequisite for detergent capsule film applications. And as Chiellini et al. [24] pointed out, in the aquatic environment (which is the relevant pathway for down-the-drain detergent products), high water solubility of PVOH is correlated with biodegradability. However, several PVOH applications outside of the detergents sector, with lower water solubility, may indeed exhibit a less favourable biodegradation profile.

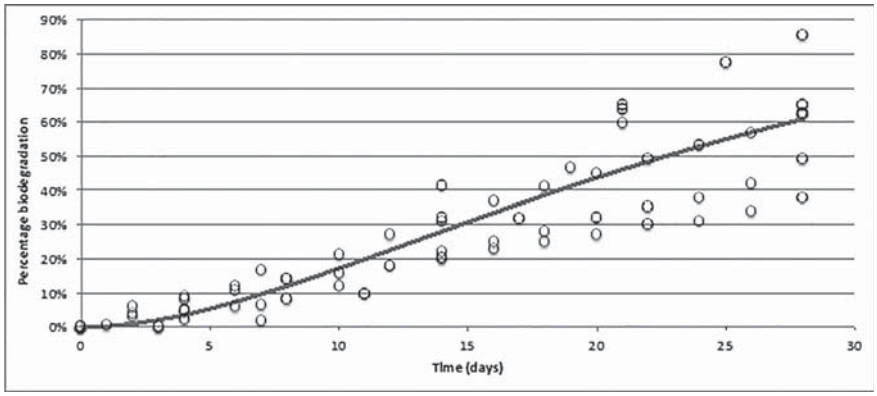

Figure 5 Biodegradation of PVOH films – aggregation of observed biodegradation data up to 28 days (circles) and the fitted logistic model (line)

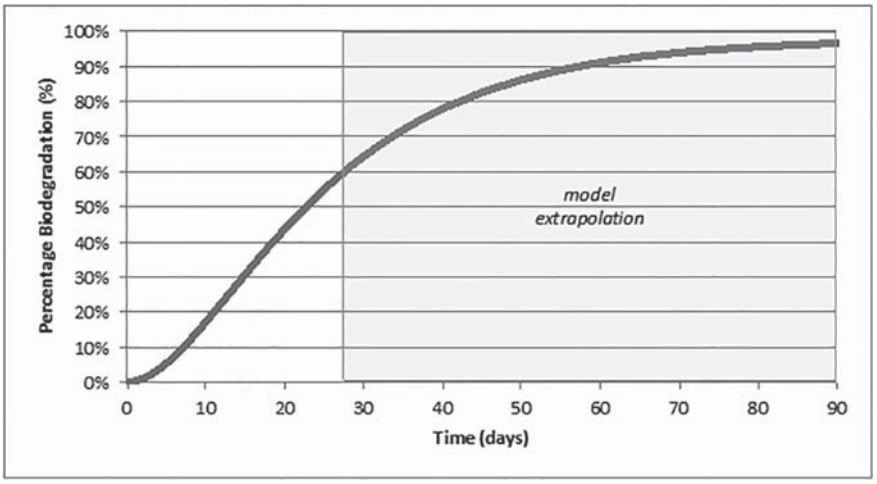

Figure 6 Biodegradation of PVOH films – model extrapolation beyond 28 days

4 Conclusions

Due to its structural similarity to biological materials such as cellulose, PVOH has the potential for complete biodegradation, especially PVOH grades that are highly water-soluble. As PVOH has a very broad diversity of applications, this unique class of polymers covers a large range of polymer chemistry and chain architecture (e.g. DH and DP, but also polymer backbone modifications). Within this range, the narrower subset of PVOH materials that is suitable for use as soluble film for detergent capsules, meets the criteria for biodegradability.

Ready biodegradability screening test data on six technical (commercial) PVOH film materials were confidentially collected, anonymised and aggregated for the current assessment. These materials represent a range of actual PVOH films, including structural modifications and added auxiliary ingredients, formulated to meet the performance and safety requirements for this specific detergents market. Substantial variability was seen between the results of biodegradation studies on different films. This includes differences in the length of the lag phase, the rate of biodegradation during the exponential phase, and the extent reached after 28 days. Some materials met the full ready biodegradation criteria. Some other materials underwent biodegradation at a lower rate and did not reach 60% biodegradation after 28 days. Nevertheless, for these materials the biodegradation process had continued and this threshold was well exceeded later on in the enhanced part of the biodegradation test (running for an additional 32 days). Modelling across all aggregated data, a total extent of biodegradation of 60% was reached after 28 days. The 10-day window is irrelevant due to the fact that the films are chemical mixtures, and because it has no relevance for the microplastic assessment.

The ready biodegradability data on PVOH films used for detergent applications, as collected and aggregated herein, confirm the information in the literature that highly soluble PVOH biodegrades in an aqueous environment. Addressing the main question of the current paper, PVOH film of detergent capsules was shown to be biodegradable in OECD screening test conditions. Despite variable biodegradation rates between studies and materials, all films were found to meet the criteria for biodegradability in these stringent studies – which offers definitive and conclusive evidence of their actual biodegradability in the environment.

In conclusion, PVOH used in liquid detergent capsule films does not meet any of the definitions of microplastic: (1) it is not micro- or nano-sized; (2) it is highly water-soluble; and (3) it is biodegradable in the environmental conditions where it is discharged.

References

1. *ECHA*: ANNEX XV RESTRICTION REPORT. Proposal for a restriction. Substance name(s): intentionally added microplastics. 22 August 2019. European Chemicals Agency, Helsinki, Finland; https://echa.europa.eu/documents/10162/05bd96e3-b969–0a7c-c6d0–441182893720.
2. *EU Commission*: Request to the European Chemicals Agency to prepare a restriction proposal conforming to the requirements of Annex XV to REACH. 2017; https://echa.europa.eu/documents/10162/13641/microplastics_cion_reqst_axvdossier_en.pdf/5c8be037–3f81–266a-d71b-1a67ec01cbf9.
3. *ECHA RAC*: Opinion of the Committee for Risk Assessment and Opinion of the Committee for Socio-economic Analysis on an Annex XV dossier proposing restrictions of the manufacture, placing on the market or use of a substance within the EU. 11 June 2020. European Chemicals Agency, Helsinki, Finland; https://echa.europa.eu/documents/10162/23665416/rest_microplastics_opinion_rac_16339_en.pdf/b4d383cd-24fc-82e9-cccf-6d9f66ee9089.
4. *Thompson, A.*: From Fish to Humans, A Microplastic Invasion May Be Taking a Toll. www.scientificamerican.com September 4, 2018.
5. *GESAMP*: Sources, fate and effects of microplastics in the marine environment: a global assessment (Kershaw, P. J., ed.). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 90, 96 p, 2015.
6. *Hidalgo-Ruz, V., Gutow, L., Thompson, R. C.* and *Thiel, M.*: Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environmental Science & Technology, *46*(6)(2012). pp 3060–3075;. PMid:22321064; DOI:10.1021/es2031505
7. *Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A.* and *Da Ros, L.*: Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuarine, Coastal and Shelf Science *130* (2013) 54–61. DOI:10.1016/j.ecss.2013.03.022
8. *Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K.* and *Janssen, C. R.*: Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Marine Pollution Bulletin, *62*(10) (2011). pp 2199–2204. PMid:21802098; DOI:10.1016/j.marpolbul.2011.06.030
9. *ASTM*: Standard Safety Specification for Liquid Laundry Packets, ASTM F3159–15e1, ASTM International, West Conshohocken, PA, 2015, www.astm.org.
10. *Finch, C. A.* (Ed.): Polyvinyl alcohol, properties and applications. Wiley, New York, 1973. 622 pp. DOI:10.1002/pol.1974.130120212
11. *Grand View Research*: Polyvinyl Alcohol (PVA) Market Size, Share & Trends Analysis Report By End Use (Paper, Food Packaging, Construction, Electronics), By Region, And Segment Forecasts, 2019–2025. (2019) https://www.grandviewresearch.com/industry-analysis/polyvinyl-alcohol-market.
12. *Thong, C. C., Teo, D. C. L.* and *Ng, C. K.*: Application of polyvinyl alcohol (PVA) in cement-based composite materials: A review of its engineering properties and microstructure behavior. Construction and Building Materials *107* (2016), 172–180. DOI:10.1016/j.conbuildmat.2015.12.188

13. *Muppalaneni, S.* and *Omidian, H.*: Polyvinyl Alcohol in Medicine and Pharmacy: A Perspective. J Develop Drugs 2 (2013) 112. DOI:10.4172/2329-6631.1000112
14. *Gaaz, T. S., Sulong, A. B., Akhtar, M. N., Kadhum, A. A. H., Mohamad, A.B.* and *Al-Amiery A.A.*: Properties and Applications of Polyvinyl Alcohol, Halloysite Nanotubes and their nanocomposites. Molecules *20* (2015), 22833 – 22847. PMid:26703542; DOI:10.3390/molecules201219884
15. *American Carp Society*: The use of PVA in carp angling (2019). https://americancarpsociety.com/pva-bags.
16. *Monosol*: Water soluble film, packets employing the film, and methods of making and using same. World Intellectual Property Organisation, publication nr WO 2016/160116 A1 (2016).
17. *Amann, M.* and *Minge, O.*: Biodegradability of Poly(vinyl acetate) and Related Polymers. Advances in Polymer Science *245* (2012) 137 – 172. DOI:10.1007/12_2011_153
18. *Julinová, M., Vaňharová, L.* and *Jurc?a, M.*: Water-soluble polymeric xenobiotics – Polyvinyl alcohol and polyvinylpyrrolidon – And potential solutions to environmental issues: A brief review. Journal of Environmental Management *228* (2018) 213 – 222. PMid:30223180; DOI:10.1016/j.jenvman.2018.09.010
19. *Chiellini, E., Corti, A., D'Antone, S.* and *Solaro, R.*: Biodegradation of poly (vinyl alcohol) based materials. Progress in Polymer Science *28*(6) (2003), 963 – 1014. DOI:10.1016/S0079-6700(02)00149-1
20. *Kawai, F.* and *Hu, X.*: Biochemistry of microbial polyvinyl alcohol degradation. Applied Microbiology and Biotechnology *84*(2) (2009), 227 – 237. PMid:19590867; DOI:10.1007/s00253-009-2113-6
21. *Solaro, R., Corti, A.* and *Chiellini, E.*: Biodegradation of poly(vinyl alcohol) with different molecular weights and degree of hydrolysis. Polym Adv Technol *11* (2000):873 – 878. DOI:10.1002/1099-1581(200008/12)11 :8/12<873::AID-PAT35>3.0.CO;2-V
22. *Yamatsu, A., Matsumi, R., Atomi, H.* and *Imanaka, T.*: Isolation and characterization of a novel poly(vinylalcohol)-degrading bacterium, Sphingopyxis sp. PVA3. Appl Microbiol Biotechnol *72* (2006), 804 – 81. PMid:16583228; DOI:10.1007/s00253-006-0351-4
23. *Wilkes, R. A.* and *Aristilde, L.*: Degradation and Metabolism of Synthetic Plastics and Associated Products by Pseudomonas spp.: Capabilities and Challenges. Journal of Applied Microbiology *123*(3) (2017), 582 – 593. PMid:28419654; DOI:10.1111/jam.13472
24. *Chiellini, E., Corti, A., Del Sarto, G.* and *D'Antone, S.*: Oxo-biodegradable polymers – effect of hydrolysis degree on biodegradation behaviour of poly(vinyl alcohol). Polym. Degrad. Stab. *91* (2006), 3397 – 3406. DOI:10.1016/j.polymdegradstab.2006.05.021
25. *Hatanaka, T., Asahi, N.* and *Tsuji, M.*: Purification and characterization of a poly(vinyl alcohol) dehydrogenase from Pseudomonas sp. 113P3. Biosci. Biotechnol. Biochem. *59* (1995), 1813 – 1816. DOI:10.1271/bbb.59.1813
26. *Chiellini, E., Corti, A.* and *Solaro, R.*: Biodegradation of poly(vinyl alcohol) based blown films under different environmental conditions. Polymer Degradation and Stability *64*(2) (1999), 305 – 312. DOI:10.1016/S0141-3910(98)00206-7
27. *Pajak, J., Ziemski, M.* and *Nowak, B.*: Poly(vinyl alcohol) – biodegradable vinyl material. Chemik *64* (2010), 523 – 530.
28. *OECD*: Guideline for testing of chemicals 302B. Zahn-Wellens/EMPA Test. July 1992. DOI:10.1787/9789264070387-en
29. *Matsumura, S., Kurita, H.* and *Shimokobe, H.*: Anaerobic biodegradability of polyvinyl alcohol. Biotechnol. Lett. *15*(7) (1993), 749 – 754. DOI:10.1007/BF01080150
30. *Marušincová, H., Husárová, L., R?žička, J., Ingr, M., Navrátil, V., Buňková, L.* and *Koutny, M.*: Polyvinyl alcohol biodegradation under denitrifying conditions. International Biodeterioration & Biodegradation *84*(2013), 21 – 28. DOI:10.1007/BF01080150
31. *Chiellini, E., Corti, A., Politi, B.* and *Solaro, R.*: Adsorption/Desorption of Polyvinyl Alcohol on Solid Substrates and Relevant Biodegradation. Journal of Polymers and the Environment (2000) *8*, 67 – 79. DOI:10.1023/A:1011569920349
32. *US EPA*: Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11, 2012. United States Environmental Protection Agency, Washington, DC, USA.
33. *Meier, F., Stelter, N., Lee, D. M., Zeese, N. J.* and *Tolls, J.*: Raw material supplier and detergent manufacturer cooperate in environmental safety assessment of a new detergent raw material – a case study. SOFW Journal *139*(3) (2013), 58 – 62.
34. *ECHA*: Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0 July 2017. European Chemicals Agency, Helsinki, Finland. DOI:10.2823/124801
35. *ECHA*: Guidance on information requirements and chemical safety assessment. Chapter r.7b: Endpoint specific guidance. Version 4.0 June 2017. European Chemicals Agency, Helsinki, Finland; https://doi.org/10.2823/84188.
36. *Reuschenbach, P., Pagga, U.* and *Strotmann, U.*: A critical comparison of respirometric biodegradation tests based on OECD 301 and related test methods. Water Res. *37*(2003), 1571 – 1582. DOI:10.1016/S0043-1354(02)00528-6
37. *Larson, R. J., Hansmann, M. A.* and *Bookland, E. A.*: Carbon oxide recovery in ready biodegradation tests: mass transfer and kinetic consideration. Chemosphere *33*(1996), 1195 – 1210. DOI:10.1016/0045-6535(96)00253-6
38. *CSTEE*: Proposed "ready biodegradability" approach to update detergents legislation. E. A. T. E. C. Scientific Committee On Toxicity, 1999.

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Bibliography
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Water soluble polymer biodegradation evaluation using standard and experimental methods

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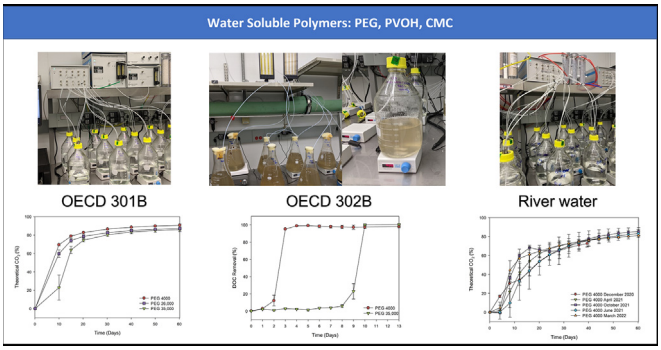
HIGHLIGHTS

- PEGs and PVOHs completely mineralized but different test durations were needed.
- CMC mineralization decreased with increasing DS.
- OECD 302 showed similar biodegradation results vs 301 but in a shorter timeframe.
- Modifications were employed to accurately evaluate mineralization.

GRAPHICAL ABSTRACT



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ABSTRACT

Multiple polyethylene glycol (PEG) polymers ranging in molecular weight (MW) from 4000 to 500,000 Da, polyvinyl alcohol (PVOH) polymers with degrees of hydrolysis (DH) of 79 % and 88 % and MW 10,000 to 130,000 Da, and carboxy methyl cellulose (CMC) polymers with degrees of substitution (DS) ranging from 0.6 to 1.2 were evaluated in standard screening biodegradation tests to assess method limitations, modification potential, and reproducibility. All PEGs and PVOHs mineralized completely in OECD 301B and 302B studies reaching >80 % biodegradation with negligible dissolved organic carbon remaining at study completion. For high MW PEOs, extension of test duration was needed to reach full extent of mineralization. CMC biodegradation was directly correlated to degree of substitution with CMC 0.6 biodegrading extensively, CMC 0.79 partially biodegrading, and CMC 1.2 not biodegrading significantly in OECD 301B and 302B studies. For all materials tested in both an OECD 301B and 302B, fewer days were necessary to reach 60 % biodegradation in the OECD 302B indicating increased rates of biodegradation with higher inoculum to test chemical ratios. In a series of investigative studies using respirometry as the analytical endpoint, significant variability in the presence of competent degraders in small volume grab samples of river water was observed. Research is needed to overcome this variability and develop a standardized reproducible test method to accurately assess polymer mineralization in river water. At study completion, residual dissolved organic carbon (DOC) data confirmed respirometry data, high levels of mineralization resulted in negligible residual DOC while low levels of mineralization resulted in significant residual DOC, up to dose concentrations. DOC measurements provided confirmation of complete biodegradation when biomass incorporation and test system set up resulted in variable carbon dioxide production or oxygen demand.

1. Introduction

Biodegradability is an important attribute for chemicals disposed of down the drain. These chemicals move through a sewer system into a wastewater treatment plant (WWTP). For soluble chemicals, any portion

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that is not biodegraded in the sewer or WWTP will enter surface waters where further biodegradation can occur. Standard methods have been developed and codified into regulations for evaluation of biodegradation of these chemicals. The standard ready and inherent biodegradation tests of the Organisation for Economic Co-operation and Development (OECD 301, 306 & 310, and OECD 302B & C) detect biodegradation in highly controlled test systems with specific buffered pH, temperature, and nutrient levels (OECD, 1992a; OECD, 1992b; OECD, 1992c; OECD, 2014). The test material is the sole carbon and energy source for a consortium of environmental microbes. These microbes are collected from relevant environmental compartments, frequently activated sludge processes of wastewater treatment plants. Relative to typical environmental conditions, the test material is dosed at a high concentration while the number of microbes present is low compared to actual wastewater treatment plants where microbial densities as evidenced by plant total suspended solids typically range from ~2000–3000 mg/L, making mineralization rates from these tests not environmentally relevant. In the screening and inherent tests, biodegradation is measured by a nonspecific analytical method either production of carbon dioxide (CO_2), consumption of oxygen (O_2), or loss of dissolved organic carbon (DOC). When initially created, the standard screening biodegradation tests were designed to test a single constituent, low molecular weight test material. However, they have been successfully applied to mixtures of structurally similar homologs, namely surfactants and oils (OECD, 2006), water soluble polymers (Van Ginkel and Gayton, 1996; Federle and Itrich, 2006; West et al., 2007; Bernhard et al., 2008), and microparticles (McDonough et al., 2017).

Ready and inherent biodegradation tests are important for persistence assessments due to their relative ease to conduct (based on lab availability, analytical needs, time, and cost) compared to simulation or field studies, which require precise analytical methods and often radiolabelled test materials. Historic work with small MW molecules has quantified relationships between ready, inherent and simulation studies (Boethling et al., 1995; Struijs and van den Berg, 1995). Based on these established relationships, regulatory environmental exposure modelling for chemicals disposed of down the drain regularly use the results of a screening study and apply a default biodegradation rate constant to assess removal during wastewater treatment and degradation in receiving compartments such as river water. For example, under REACH regulations, chemicals that are ready biodegradable are given default first order biodegradation rates in wastewater treatment (1.0 h^{-1}) and river water (2 × 10^{-3} h^{-1}) (ECHA, 2003).

Research has highlighted the limitations of the standard screening and inherent tests for evaluation of chemical persistence due to the conservative nature of the test systems. Kowalczyk et al. (2015) thoroughly reviewed the ready and inherent test method limitations and discussed inoculum source and preparation variations, inoculum levels, high test chemical concentrations, and test duration as key concerns. Due to the limitations of stringent screening studies, ECHA has created an enhanced ready biodegradability testing strategy (eRBT) in order to leverage data from OECD 301 studies. The enhancements include test extension from 28 to 60 d and use of larger test vessels, allowing increased microbial diversity in the test system while maintaining the test substrate to inoculum ratio prescribed in the test guideline (TG) (ECHA, 2014; ECHA, 2017). Recent research showed that test extension and larger test vessels were suitable enhancements and reliable results from eRBT studies were obtained for 5 structurally different test compounds with varied biodegradation profiles (Gartiser et al., 2022). Separate studies found that increased test duration and inoculum levels led to improved reproducibility of results from OECD 301 studies (Martin et al., 2017).

Recently, significant focus has turned to the development of biodegradable polymers for use in down-the-drain products. In addition, there is renewed regulatory focus on methods for assessment of polymer biodegradation as the EU Commission evaluates new approaches to register polymers under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Therefore, it is important to probe the ability of standard assessment tools to reliably quantify polymer biodegradation. For this research three classes of water-soluble polymers were evaluated: polyethylene glycols (PEG), polyvinyl alcohols (PVOH), and carboxy methyl celluloses (CMC). All are commonly used in products that are disposed of down the drain. Further, a range of polymers within each main class were evaluated to gain a fundamental understanding of the impact of polymer structure on biodegradation. PEG materials were chosen due to the simplicity of their structure i.e., repeating units with minimal branching or other substitutions and their availability in a wide range of average molecular weights (MW). At higher MWs, the synthesis route of these polymers alters and alkyl initiators such as tertbutyl are used resulting in an endcap at one end of the molecule (Herzberger et al., 2016). The effect of this end group on biodegradability is important to elucidate. In this study, test materials with a tertbutyl endcap are termed polyethylene oxide (PEO). The next class of materials chosen for evaluation in this research is PVOH. PVOH polymers are formed by the hydrolysis of polyvinyl acetate and are characterized by their varying degrees of hydrolysis (DH) and MW, allowing evaluation of the impacts of these structural components on biodegradation. The final class of polymers chosen was CMC. CMCs have varying degrees of carboxy methyl substitution (DS) on a cellulose backbone allowing evaluation of the impact of substitution level on biodegradation.

The objectives of this research were to systematically evaluate the biodegradability of a suite of water-soluble polymers from different polymer classes by leveraging and modifying standard screening (OECD 301B) and inherent (OECD 302B) biodegradation test methods. Modifications to test systems included extension of the test duration and the addition of other nonspecific analytical endpoints beyond standard requirements. Trends observed across different analytical endpoints, between different test systems, and across polymer classes were probed to elucidate test method applicability and the impact of test design on results. Preliminary research explored the use of these same analytical endpoints to evaluate variability in the presence of competent degraders when grab samples of river water were used as both the test system inocula and test media. Variability in the presence of competent degraders was evaluated during each study as well as at multiple sample points across a 2-yr period and in two separate geographic locations (US and Europe).

2. Material and methods

2.1. Test materials

The test materials included PEG and PEO materials ranging in MW from 4000 to 500,000 Da, PVOH materials ranging in MW from 10,000 to 130,000 Da with DH of 79 % and 88 %, and CMC materials ranging in approximate MW from 210,000 to 250,000 Da with DS of 0.6, 0.79, and 1.2. The specific materials are the following: PEG 4000 (Sigma BioUltra), PEG 26,000 (Polymer Standards Service), PEG 35,000 (Sigma BioUltra), PEO 30,000 (Polymer Standards Service), PEO 50,000 (Agilent), PEO 100,000 (Agilent), PEO 300,000 (Agilent), PEO 500,000 (Agilent), PVOH79 (Sigma; DH 79 %, MW 9–10,000 Da), Mowiol 4–88 (Sigma, hereafter termed PVOH 4–88, MW = 31,000 Da, note 4 refers to average viscosity and 88 refers to average DH, see Table S2), Mowiol 8–88 (Sigma, hereafter termed PVOH 8–88, MW = 67,000 Da), and Mowiol 18–88 (Sigma, hereafter termed PVOH 18–88, MW = 130,000 Da), CMC 0.6 (DS = 0.6, TCI America), CMC 0.79 (DS = 0.79, Sigma), and CMC 1.2 (DS = 1.2, Sigma). Test material characterization information are provided in Tables S1–S3. The tests also required a positive control, sodium benzoate (Alfa Aesar) or microcrystalline cellulose (Merck); a phosphate buffer (KH_2PO_4, Acros Organics; K_2HPO_4, Acros Organics; $Na_2HPO_4 \times 2H_20$, Merck); and nutrient salts (ammonium chloride, Alfa Aesar; calcium chloride 2.75 % *w/v*, BDH; magnesium sulphate 2.25 % w/v, BDH; and iron (III) chloride 0.025 % w/v, BDH). All laboratory water used in this study was obtained from a Milli-Q Advantage A10 (Millipore).

2.2. OECD 301B ready biodegradability test

Studies were conducted following the OECD 301B Ready Biodegradation test guideline (OECD, 1992) on the entire suite of polymers. The 1 L test systems consisted of phosphate buffered media (pH = 7.1) with

nutrient salts and activated sludge inoculum dosed to 17 mg/L total suspended solids (TSS). The activated sludge was collected 3 d prior to test initiation from a WWTP receiving >90 % domestic sewage (Fairfield, OH) and aerated in the lab until use. On the day of test initiation, the activated sludge inoculum was washed twice with media, homogenized in a blender, passed through a 2 mm sieve, and the TSS subsequently adjusted to 1700 mg/L. Test material stock solutions (1 g/L) were analyzed for carbon content on a total organic carbon (TOC) analyzer (Shimadzu Scientific Instruments, Columbia, MD) and dosed into each system at 12–14 mg C/L (additional test setup information provided in Table S4). Duplicate or triplicate test systems were incubated with stirring at 21.5 °C ± 0.5 in the dark for the duration of the test. Sodium benzoate was used as the positive control to confirm the viability of the inoculum and an inoculum blank control was used to measure background respiration. The test vessels were monitored by a MicroOxymax System (Columbus Instruments, Columbus, OH). At each sample point, the MicroOxymax system measured the percent of CO_2 in the headspace of each test system using an infrared detector. At test initiation and after each analysis the headspace and gas detector were purged and refreshed with air that had been scrubbed of CO_2 using soda lime. Therefore, any measured CO_2 was evolved from the microbial activity in the test system. CO_2 above background respiration indicated mineralization of the test material. Note: significant prework was conducted to optimize the test setup and align on 17 mg/L TSS and 12–14 mg C/L test substance addition in order to ensure a signal to noise ratio of 3:1 for the quantification of CO_2 evolution for the test substance above the background respiration. The test systems were incubated until the mineralization of all test materials had reached a plateau. At test termination the test systems were acidified to pH < 3 and evaluated for residual dissolved organic carbon (DOC).

2.3. OECD 302B Zahn Wellens test

Inherent biodegradation tests were conducted following the OECD 302B Zahn Wellens test guideline (OECD, 1992). The test system consisted of phosphate buffered media (pH = 7), nutrient salts, activated sludge inoculum dosed at 200 mg/L TSS, and test material dosed at 50 mg C/L. TOC (Shimadzu) of each stock solution (2 g/L) was quantified and used to calculate dose volume. In order to correspond to the OECD 301B studies, sodium benzoate was used as the positive control to confirm the viability of the inoculum and an inoculum blank control was used to measure background DOC or CO_2 evolution depending on the analytical endpoint. Duplicate test systems were incubated with stirring at 21.0 °C ± 1 °C in the dark (additional test setup information in Table S5). For PEG 4000; PEG 35,000; PEO 50,000; PEO 100,000; CMC 0.6; and CMC 0.79 biodegradation was measured as the loss of DOC from the test media. At each sample point 50 mL were removed from each test system, centrifuged at 1500 ×*g* for 15 min, and supernatant DOC quantified. The first sample point was collected 3 h after dosing to ensure no loss of test chemical via adsorption to activated sludge solids (by comparison of the 3 h time point with the dosed carbon, Table S6). Thereafter the remaining DOC was expressed as a percentage of the initial 3 h measurement. To automate the data collection from a 302B and to directly quantify test substance mineralization via CO_2 evolution test vessels containing PVOH79; PVOH 4–88; PVOH 8–88; PVOH 18–88; CMC 0.6; CMC 0.79; and CMC 1.2 were monitored using the MicroOxymax System and the method outlined previously for the OECD 301B head space CO_2 analysis. For these systems, residual DOC was quantified at test termination.

2.4. Biodegradation tests using river water

United States river water was collected from the bank of the Great Miami River (GMR) in Colerain, OH (39°17′27.45″N, 84°39′46.70″W or 39°15′47.31″N, 84°41′21.58″W, depending on accessibility). 1 L aliquots of the river water were used on the day of collection as both the test media and microbial inoculum (autochthonous river water flora used as test system inoculum) with no alteration other than the addition of nutrient salts dosed to the levels prescribed in the OECD 301 (OECD, 1992). Biodegradation was monitored as described above (Section 2.2). Additional test setup information is provided in Table S7.

In Europe, Charles Rivers Laboratory (Den Bosch, Netherlands) conducted a respirometry study using Dutch river water collected from two locations along the Meuse River (MR) in 's-Hertogenbosch, (51°44′39.1″N 5°12′54.3″E and 51°44′20.6″N 5°17′00.4″E). The samples were combined in equal volumes and used as a single inoculum. The inoculum was passed through 1 mm sieve to remove any large sediment and debris, amended with nutrient salts, and then subdivided into 238 mL aliquots and oxygen consumption was monitored for a seven-day preincubation period under test conditions prior to test material dosing in order to reduce background respiration levels. Test vessels exhibiting higher or lower than average respiration were not used in the subsequent test in order to reduce variability due to background respiration. Biodegradation was measured as oxygen demand (OD). The test material stocks solutions were analyzed for TOC to confirm the dose concentration. The test materials were dosed at 100 mg/L, corresponding to 96–172 mg OD/L and 40–60 mg C/L (additional test setup information in Table S8). Inoculum blanks and positive controls (microcrystalline cellulose) were run in parallel. Each test treatment was evaluated in quadruplicate. The test systems were attached to a Lovibond BD600-GLP Plus manometric respirometry system and incubated with stirring at 21.8–22.7 °C in the dark. Evolved CO_2 was absorbed by soda lime pellets and the resulting drop in pressure was converted to amount of consumed oxygen. O_2 consumption greater than background respiration was quantified as mineralization. At test termination residual DOC was quantified. Table S9 provides detailed information on river water at the time of collection including flow, temperature, pH, suspended solids, ammonia and COD and final test system pH.

2.5. CFU determination

For some GMR samples, the number of colony forming units (CFU/mL) was quantified following ISO 8199 spread plate technique (ISO, 2018). On the day of river water collection, aliquots of river water were serially diluted in HQW. Volumes of 100 μL were plated onto previously prepared nutrient agar plates (BD-Difco). Each dilution was plated in duplicate with a water control. The plates were inverted and incubated at 22 ± 2 °C for 24 ± 3 h. Colonies were then manually counted.

3. Results and discussion

3.1. OECD 301B ready biodegradability test

The results of the OECD 301B studies using activated sludge inoculum are summarized in Fig. 1 and Tables 1 and S10. In all studies, the positive control, sodium benzoate, reached >60 % theoretical CO_2 evolution ($ThCO_2$) by 14 d indicating inoculum viability and meeting test validity criteria (Fig. S1). In all studies the inoculum blanks reached <40 mg/L CO_2 evolution at 28 d and over the duration of the study thus meeting validity criteria. The low MW PEG and PEO materials (average MW ≤ 35,000) biodegraded rapidly reaching >70 % $ThCO_2$ in 28 d and >80 % $ThCO_2$ production in 60 d. Within the three PEG materials, the lag time to reach 10 % increased from 4 to 9 d with increasing average MW from 4000 to 35,000 Da. The PEO 30,000 had a lag of 12 d, which is longer than the PEG 35,000 indicating that the terminal tertbutyl group slowed but did not shut down biodegradation. This observation can be explained by the previously established PEG biodegradation pathway that progresses through sequential oxidation of the glycol units (Kawai and Yamanaka, 1986; Sparham et al., 2008) and requires at least one terminal hydroxyl group (Beran et al., 2013) for biodegradation to occur. Although not required by the OECD 301B TG (OECD, 1992), the evaluation of DOC remaining at study completion can provide valuable information on the level of residual parent test compound or metabolites. For the low MW PEG and PEO materials (average MW ≤ 35,000 Da), negligible DOC (<4.5 %) remained at study completion indicating complete mineralization of the

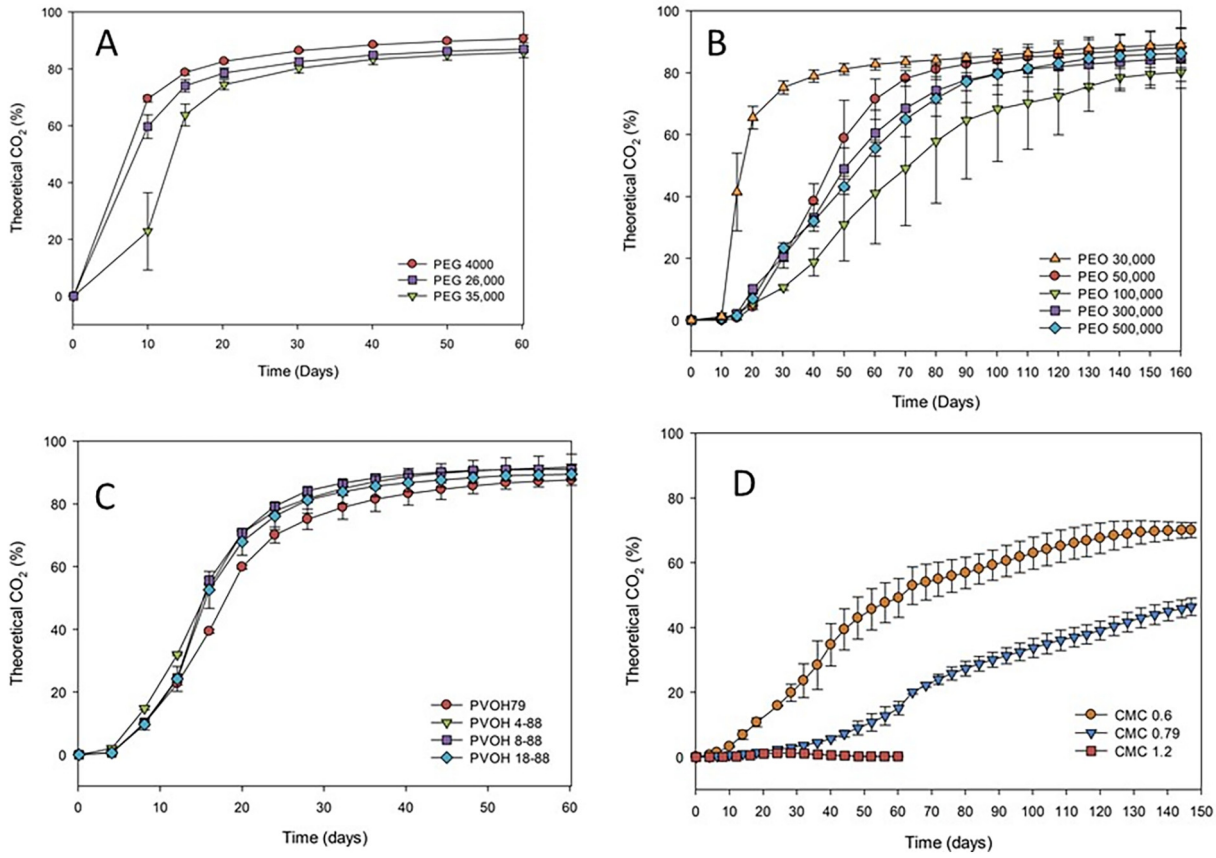

Fig. 1. Biodegradation of water-soluble polymers in an OECD 301B using activated sludge inoculum A) PEG materials B) PEO materials C) PVOH materials D) CMC materials. Presented as an average of replicate test systems ($n \geq 2$) with error bars showing standard deviation.

Table 1
Percent of dosed carbon remaining as dissolved organic carbon (DOC) at test termination.

Test material	301B		302B		GMR-RW		MR-RW	
	Test duration (d)	DOC (%)	Test duration (d)	DOC (%)	Test duration (d)	DOC (%)	Test duration (d)	DOC (%)
PEG 4000	160	2.9 ± 2.1	13	2.1 ± 0.70	39	0	180	2.6
					48	2.3 ± 0.65	180	58
					89	0	180	3.8
					62	0	180	4.2
					98	0.61 ± 0.32		
PEG 26,000	160	3.8 ± 1.4			48	2.6 ± 0.61		
					89	88 ± 0		
PEG 35,000	160	3.2 ± 2.0	13	0 ± 0	39	0	180	87
					48	67 ± 17		
					89	94 ± 2.8		
					62	89 ± 1.8		
PEO 30,000	160	4.4 ± 1.2			48	88 ± 6.1		
					89	97 ± 1.2		
PEO 50,000	160	4.6 ± 4.6	60	3.5 ± 0.58				
PEO 100,000	160	7.2 ± 0.89	60	2.2 ± 1.3				
PEO 300,000	160	5.2 ± 5.1						
PEO 500,000	160	3.4 ± 1.6						
PVOH79	60	2.0 ± 2.4	28	3.7 ± 0.36	89	1.4 ± 0.38	180	72
							180	71
							180	0
							180	54
PVOH 4-88	60	3.5 ± 0.66	28	4.5 ± 1.5				
PVOH 8-88	60	4.3 ± 0.70	28	3.6 ± 0.77				
PVOH 18-88	60	4.1 ± 0.37	28	3.2 ± 0.94				
CMC 0.6	147	11 ± 0.65	100	15 ± 1.1	89	22 ± 2.3	180	13
CMC 0.79	147	40 ± 3.7	100	61 ± 2.8				
CMC 1.2	60	98 ± 2.6	60	96 ± 0.040				

polymers (Table 1). These results agree with published literature where nine PEG polymers ranging in MW from 250 to 58,000 Da were evaluated in a DOC removal test (Bernhard et al., 2008). In this study, all PEGs MW ≤ 14,600 Da reached >80 % DOC removal in 28 d and PEG 26,600 was slower than in the current study and but still reached >80 % DOC removal in 60 d.

All high MW PEOs (MW ≥ 50,000 Da) had lags of 20–29 d, but once mineralization started, it progressed steadily. PEO 50,000 reached 71 ± 6.5 % $ThCO_2$ in 60 d, while PEO 100,000 reached 41 ± 16 % $ThCO_2$, PEO 300,000 reached 61 ± 7.4 % $ThCO_2$ and PEO 500,000 reached 56 ± 2.6 % $ThCO_2$ (Fig. 1B, Table S10). The PEO 100,000 was more variable than the other test materials due to a single, lagging replicate (Fig. S2). To evaluate full potential for mineralization of all replicates, the test duration was extended to 160 d to allow this lagging replicate to reach >80 % $ThCO_2$. Generally, residual DOC at test termination was very low for PEOs (<5.2 %), but slightly higher for PEO 100,000 (7.2 %) due to the slower replicate as discussed above. The test replicate variability could be attributed to the OECD 301B test set-up resulting in a microbial lottery where an abundance of competent degraders may not initially be present in sufficient quantities, and it may take time for communities to grow to a level that can appreciably mineralize the test compound. Additionally, the longer test duration required for complete mineralization to occur in these studies can be explained by the fact that the amount of carbon dosed was held constant, and thus the number of molecules, and by extension terminal glycol units, present in the test systems dropped by 1–2 orders of magnitude (OM) as MW increased from 4000 to 500,000 Da. Thus, both MW increase and synthesis route alteration (use of tert-butyl initiator) decrease the number of locations for microbial attack in the high MW PEOs.

All PVOHs mineralized completely during the study reaching >75 % $ThCO_2$ by 28 d and >87 % $ThCO_2$ production at 60 d (Fig. 1C) with negligible DOC remaining at study completion (<4.3 % DOC), Table 1. Reproducibility was strong as evidenced by the low standard deviation among replicates. For the MW range tested (10,000–130,000 Da) no significant difference was observed in extent of mineralization (88 to 92 % $ThCO_2$ range for all PVOHs evaluated) or time to reach complete mineralization. In addition, no significant impact on mineralization was noted for the sample with a lower DH (79 %) as compared to the other PVOHs with higher DHs (88 %). Like the results observed in this study with non-adapted inoculum from a domestic activated sludge WWTP, Chiellini et al. (2003) showed that PVOH with a DH of 88 % reached >60 % $ThCO_2$ evolution in a 28 d respirometric study using adapted inoculum from a paper mill wastewater treatment plant. Similarly, high levels of mineralization, matching that of cellulose, for PVOH (DH = 72 %, MW = 9500 Da); PVOH (DH = 88 %, MW 44,900 Da), and PVOH (DH = 98 %, MW = 20,300 Da) were measured using the same adapted inoculum (Solaro et al., 2000). Finally, >5 % mineralization of films made from Mowiol 3–98, 4–88, 4–98, 10–98, and 20–98 (representing DH = 88 % and 98 % and MW ranging ~16,000–125,000 Da) was measured in a modified Sturm test using activated sludge from a domestic WWTP (Haschke et al., 1998).

CMC polymers showed decreasing levels of biodegradation with increasing DS and test extension was employed to fully evaluate potential for biodegradation (Fig. 1D, Tables 1 and S10). CMC 0.6 reached 20 ± 2.4 % $ThCO_2$ in 28 d, 49 ± 6.0 % $ThCO_2$ in 60 d, and ultimately 70 ± 2.3 % $ThCO_2$ (11 ± 0.7 % DOC remaining) at 148 d. CMC 0.79 was slower to mineralize reaching 2.9 ± 0.5 % $ThCO_2$ in 28 d, 15 ± 2.1 % $ThCO_2$ in 60 d, and 46 ± 2.7 % $ThCO_2$ in 148 d with 40 ± 3.7 % DOC remaining. No appreciable level of CO_2 production was observed for CMC 1.2 in a 60-d study with 98 % DOC remaining. By comparison, the unmodified, insoluble microcrystalline cellulose used as a positive control reached 82 ± 1 % $ThCO_2$ in 28 d with negligible residual DOC. Results from this study are consistent with results from Van Ginkel's research which measured 60 % mineralization in 110 d of a CMC with DS = 0.7 in an OECD 301D Ready Biodegradation Study following biological oxygen demand as the analytical endpoint (Van Ginkel and Gayton, 1996). Additionally, historic work showed that increasing the carboxy methyl substitution on cellulose can slow but not shut down the enzymatic hydrolysis of the glycolytic bonds with blockiness of the substitution locations also being a contributing factor to degradation and CMC DS > 1 resulting in no degradation in these enzyme studies (Saake et al., 1998).

3.2. OECD 302B Zahn Wellens test

A series of OECD 302B studies were conducted on a subset of the polymers covering each of the different polymer classes (Fig. 2, Tables 1, S11 and S12). In all tests, sodium benzoate reached >90 % DOC removal by 5 d or >60 % $ThCO_2$ by 14 d (Fig. S1). PEG and PEO polymers (PEG 4000; PEG 35,000; PEO 50,000; and PEO 100,000) fully biodegraded. PEG 4000 showed >95 % DOC removal by 4 d and PEG 35,000 had an 8-d lag but then reached >95 % DOC removal in 10 d. The two higher MW PEO materials experienced a 20-d lag and then reached >95 % DOC removal by 40 d and were statistically indistinguishable from each other. All four materials showed complete DOC removal over the course of the test but the higher MW PEOs required test extension beyond the traditional 28 days to 60 days to allow for the full extent of biodegradation to occur as evidenced by a plateau in DOC removal.

As discussed in Section 2.3, PVOH test materials were evaluated in a modified OECD 302B following evolved CO_2 as the analytical endpoint and measuring DOC remaining at test termination. All PVOH polymers mineralized completely reaching >88 % $ThCO_2$ production with negligible DOC remaining (<4.5 %) at 28 d. No significant impact of MW and DH on biodegradation rate and extent was observed in this study.

CMC 0.6 and 0.79 were evaluated both following DOC and CO_2 production to compare analytical endpoints. The analytical endpoints did not yield significant differences in results for either test material (Fig. 2D, Tables 1, S11, and S12). CMC 0.6 reached 57 % biodegradation at 28 d, 74 % biodegradation at 60 d and 88 % biodegradation in 142 d in the DOC removal study. Similarly, in the CO_2 production study, CMC 0.6 reached 84 % $ThCO_2$ evolution in 100 d. CMC 0.79 reached 12 % biodegradation at 28 days and plateaued at approximately 36 %. CMC 1.2 showed no significant mineralization to CO_2 in 60 d with 96 % DOC remaining at test termination. The strong agreement between CO_2 and DOC endpoints is likely due to the extracellular biodegradation processes dominating removal for these large MW polymers that cannot cross cell membranes. Conversely, sodium benzoate rapidly crosses the cell membrane and is thus removed from the DOC of the test system very quickly but requires several more days to be completely mineralized to CO_2 by the microorganisms then transferred from the aqueous test media to the headspace (carbonate system mass balance) and quantified by the CO_2 detector (Fig. S1).

3.3. Preliminary river water studies

A preliminary investigation was conducted using river water as a media and inoculum source to understand variability and impacts on polymer biodegradation. One of the main challenges of river water studies is the variability that has been observed in biodegradation studies due to the dilute nature of the aqueous environment combined with small grab samples used in laboratory test systems (1 L or 238 mL in this research) leading to low microbial diversity and abundance. This results in a microbial lottery as a small grab sample of river water may not contain competent degraders but the competent degraders may actually be present in the aqueous environment. In addition, respirometric methods require the use of relatively high-test chemical concentrations (many orders of magnitude higher than environmental concentrations), so if small communities of degraders are present, a significant amount of time and optimal test conditions will be needed for the degrader population to multiply to a sufficient level to mineralize the test chemical. The issue of low microbial cell concentrations leading to variable biodegradation test results has been observed for small molecules. For example, Martin et al. (2018) and Thouand et al. (1995) studied 4-nitrophenol biodegradation and showed that low microbial cell counts in surface water can lead to variation in test results using high throughput screening approaches leveraging 96 well plates. They further showed that cell concentrations at 10^6–10^8 CFU/mL are needed to accurately predict biodegradability of this compound.

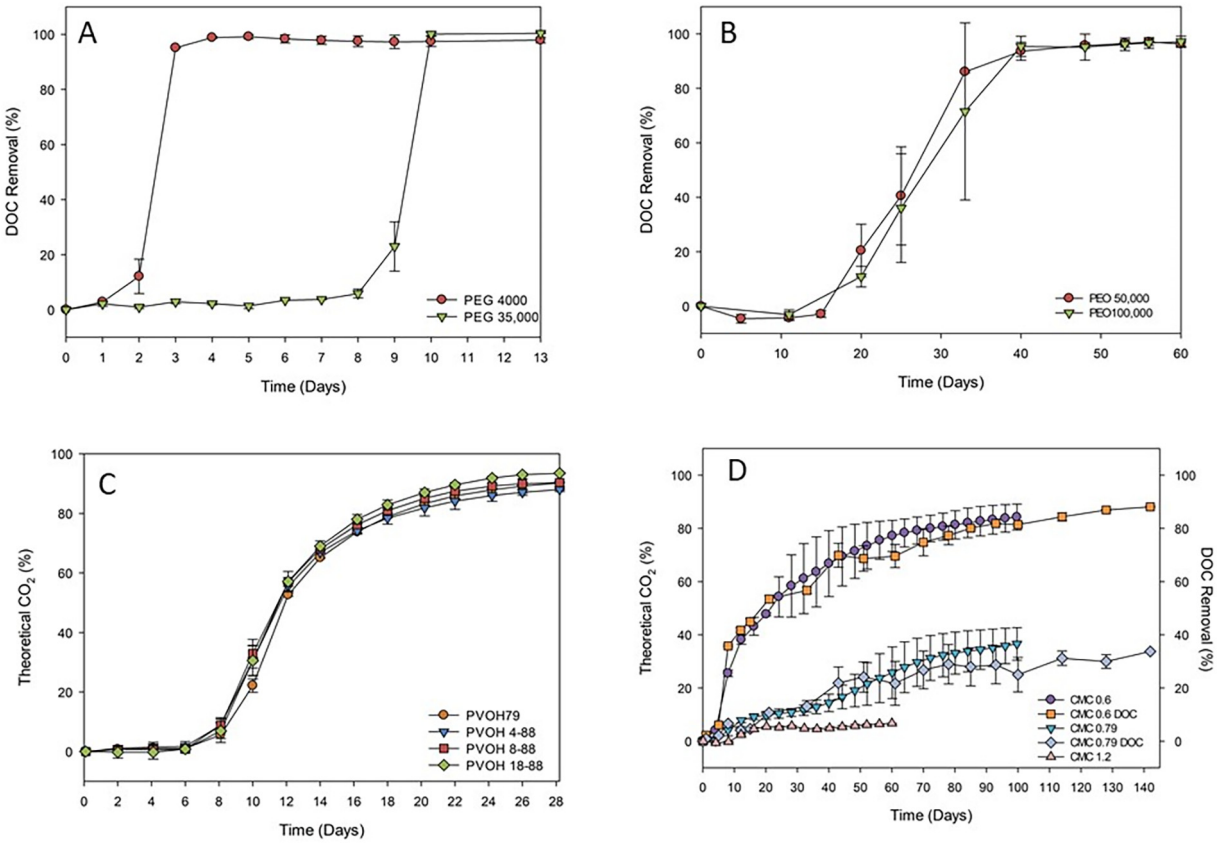

Fig. 2. Biodegradation of water-soluble polymers in an OECD 302B Modified Zahn-Wellens Test A) PEG materials B) PEO materials C) PVOH materials D) CMC materials. Presented as an average of replicate test systems ($n = 2$) with error bars showing standard deviation.

A series of respirometry studies using river water as both the media and the inoculum source were used to screen for the presence of competent degraders in river water and to dimension the variability that occurs with grab samples. This series of tests evaluated the inter and intra test system variability across seasons, geographies, and test set ups for PEG 4000; 26,000; and 35,000; PEO 30,000; PVOH79; and CMC 0.6, (Figs. 3 & 4 and Tables 1, S13, and S14). The positive controls biodegraded in all tests (Fig. S1) with sodium benzoate replicates reaching >70 % $ThCO_2$ and negligible DOC in all GMR studies and microcrystalline cellulose replicates reaching >75 % theoretical O_2 demand (ThOD) in the MR study. High inorganic carbon levels in the river water media at test termination indicate that the evolution of CO_2 into the test vessel headspace and thus the detection of mineralization was slowed by the alkalinity of the river water and the retention of produced CO_2 in the aqueous phase due to carbonate system equilibria. In general, the river water had a starting pH = 8 and at test termination pH = 9–10 (Table S9). Thus, the time to detect mineralization was likely much longer than the actual time to mineralization. pH adjustment or buffering should be considered in future test set ups to shorten test duration and accurately quantify CO_2 evolution; however, it could affect microbial activity, so additional research is needed to optimize the test design.

Mineralization of PVOH79 in GMR river water was extensive, reaching 71 ± 1.2 % $ThCO_2$ with negligible DOC remaining showing that the microbial community present in that sub sample was able to fully mineralize PVOH79. Variation in the results from each of the replicates was negligible indicating a sufficient abundance of competent degraders in each of the test systems. By comparison, the MR study showed high variability between replicates with one replicate reaching complete mineralization (79 % ThOD with negligible DOC remaining) while a second plateaued at 27 % ThOD and the remaining two replicates plateaued at 11 and 15 % ThOD, residual DOC was commensurate with mineralization (note that it is common to discard a study with replicate variation >20 % highlighting the need for better test system design to reduce variability in results). This variation indicates that the consortium of microbes present in the MR can also completely degrade PVOH79, but the multiple different microbes needed to completely mineralize PVOH (Chiellini et al., 2003) were present in some but not all of the test vessels due to the issues of variability in microbial communities when collecting grab samples of river water as discussed above. In addition, the MR inoculum was preincubated in the lab for 7 days which can also lead to a decline in microbial diversity (Vázquez-Rodríguez et al., 2011).

CMC 0.6 mineralized extensively reaching 54 ± 1.8 % $ThCO_2$ in 89 d with 22 ± 2 % DOC remaining in GMR inoculum and 79 ± 6.9 % ThOD in 180 d with 13 % DOC remaining in MR inoculum with limited variability among replicates in each test system. These consistent results imply a sufficient abundance of CMC degraders present in GMR and MR surface waters. This abundance of competent degraders may be explained by the naturally occurring presence of plant matter (cellulose) in these environmental systems.

Multiple PEG and PEO studies were conducted with GMR inoculum to evaluate the reproducibility of respirometry results. PEG 4000 was evaluated in 5 different river water grab samples collected from December 2020 through March 2022. River water characterization for each study can be found in Table S9. Mineralization of PEG 4000 was extensive in all studies reaching >70 % $ThCO_2$ with negligible DOC remaining (Fig. 3A, Table 1). Variability was observed among replicates especially during the growth phase of the mineralization curves but convergence in $ThCO_2$ was observed among replicates as the test chemical completely mineralized and the $ThCO_2$ curves plateaued. This variation in the growth phase is due to microbial variability in the grab sample as discussed above, limited numbers of competent degraders were present in some of the samples and time was needed for the degraders to multiply and reach a sufficient population level that test chemical mineralization could be quantified. Comparing the different tests, variability in average time to reach 60 % $ThCO_2$ was observed ranging from 12 to 24 d. Variability was also observed in average

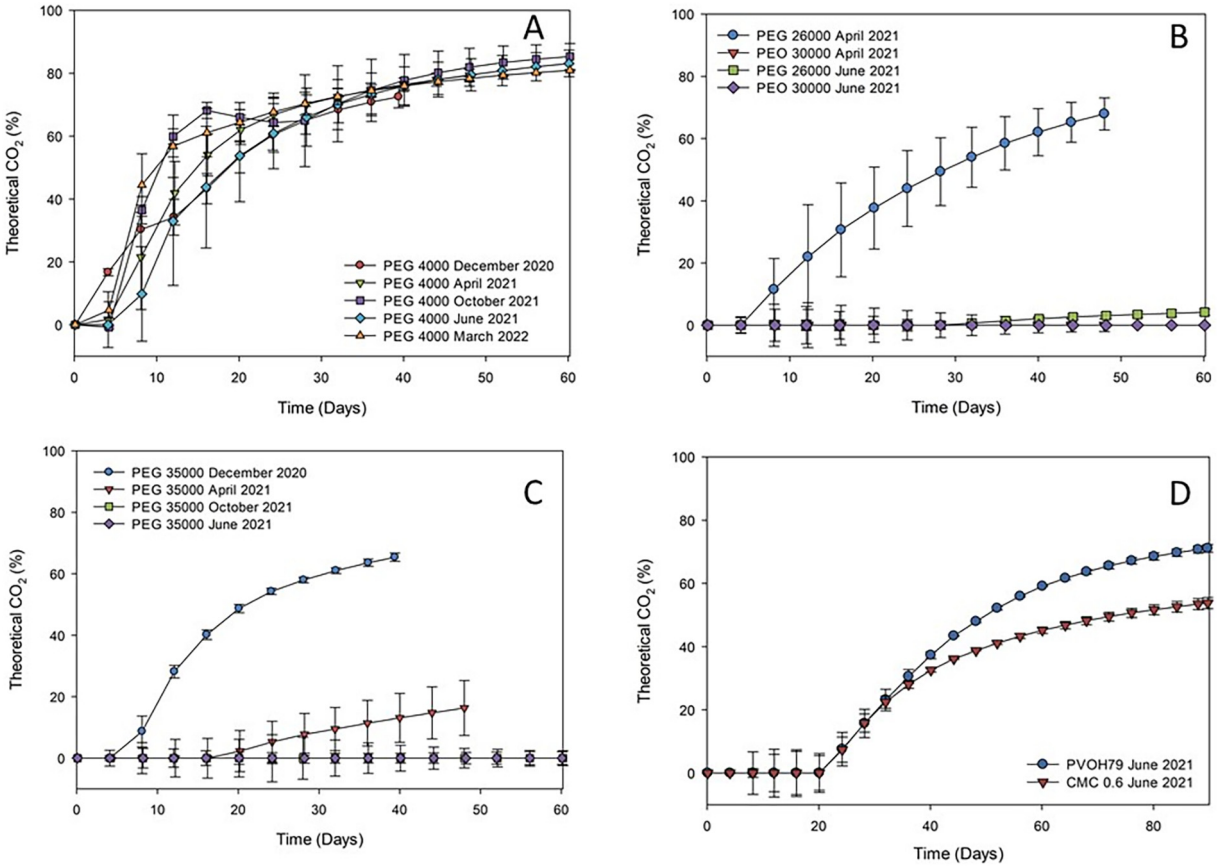

Fig. 3. Biodegradation of water-soluble polymers in a series of river water studies using GMR as media and inoculum A) PEG 4000 results from five separate studies B) PEG 26,000 and PEO 30,000 results from two separate studies C) PEG 35,000 results from four separate studies D) PVOH79 and CMC 0.6 results from one study. Presented as an average of replicate test systems ($n \geq 2$) with error bars showing standard deviation.

extent of %$ThCO_2$ detected, with the shorter duration tests having lower % $ThCO_2$ detected than the other studies but negligible DOC remaining indicating complete mineralization. As discussed above, both the duration to 60 % $ThCO_2$ and the level of final %$ThCO_2$ is highly impacted by the system pH resulting in the evolved CO_2 remaining solubilized rather than being released into the headspace (carbonate system equilibria). As seen with the PVOH results, PEG 4000 exhibited significant variability in the MR inoculum (Fig. 4A, Table S14). Significant biodegradation was seen in all four test replicates with three replicates reaching complete biodegradation (76 ± 9 % ThOD) with negligible DOC remaining (Table 1). However, the time to reach 60 % varied from 18 to 161 d, once again indicating that the available microbial community was able to biodegrade PEG 4000 but variable microbial counts, high test concentration, and test set up hampered the ability to do so consistently among replicates.

For PEG and PEO materials 26,000–35,000 significant intra and inter test variability was observed using GMR inoculum. PEG 26,000 showed little mineralization in the June 2021 GMR study and completely mineralized in the April 2021 study, but replicate variability was significant especially during the microbial growth phase. Likewise, PEG 35,000 showed little mineralization in 2 studies, variable mineralization in one study and complete mineralization in another study. Evaluation of the river water grab sample parameters showed no significant differences between the inoculum samples that could explain the observed variability in results (Table S9).

To gain additional insights into the microbial abundance in the river water grab samples, microbial counts were measured in the GMR inoculum collected in March 2022 and found the river water contained 1.1×10^3 CFU/mL. Three grab samples were again collected in July 2022 and similarly contained 10^3 CFU/mL, even as the flow ranged from 2000 to 7000 ft^3/s (Table S15). These levels are 1–2 OM lower than the level of microbial activity suggested in an OECD 301B (OECD, 1992) and 3 to 5 OM lower than suggested by Martin et al. (2018) and Thouand et al. (1995) in order to have sufficient microbial abundance and diversity needed for reproducible test results. This investigative work on river water highlights that biodegradation of polymers in river water needs further research and the observations and findings from this study should be considered preliminary. As a next step for this research, work will be undertaken to understand if microbial concentration, test set up modifications, or other improvements in biomass to test chemical ratios can ameliorate replicate and test system variability. Microbial fingerprinting will also be investigated.

3.4. Analytical endpoint analysis

For screening and inherent type studies using non-specific analytical methods it is important to evaluate what each endpoint is measuring, expected variation in these endpoints, and the implications for data analysis. In these studies, a high level of test chemical is used to ensure that the CO_2 produced, O_2 consumed, or DOC removed during the biodegradation of the test chemical are quantitatively measurable above background levels in blank controls. DOC is a measurement of the organic carbon present in the aqueous phase and therefore full extent of DOC loss can be quantified down to the limit of quantification for the test system and analyzer (it is important to highlight that the DOC endpoint can only be used for a soluble test substance, and it is critical to check for no adsorption to activated sludge in the initial time point to accurately assess biodegradation). For small molecules easily passed through the cellular membrane, this endpoint typically leads to faster quantification of biodegradation as there are not the mass transfer limitations that exist when following respirometry endpoints. CO_2 evolution is a measure of the portion of the carbon in the test

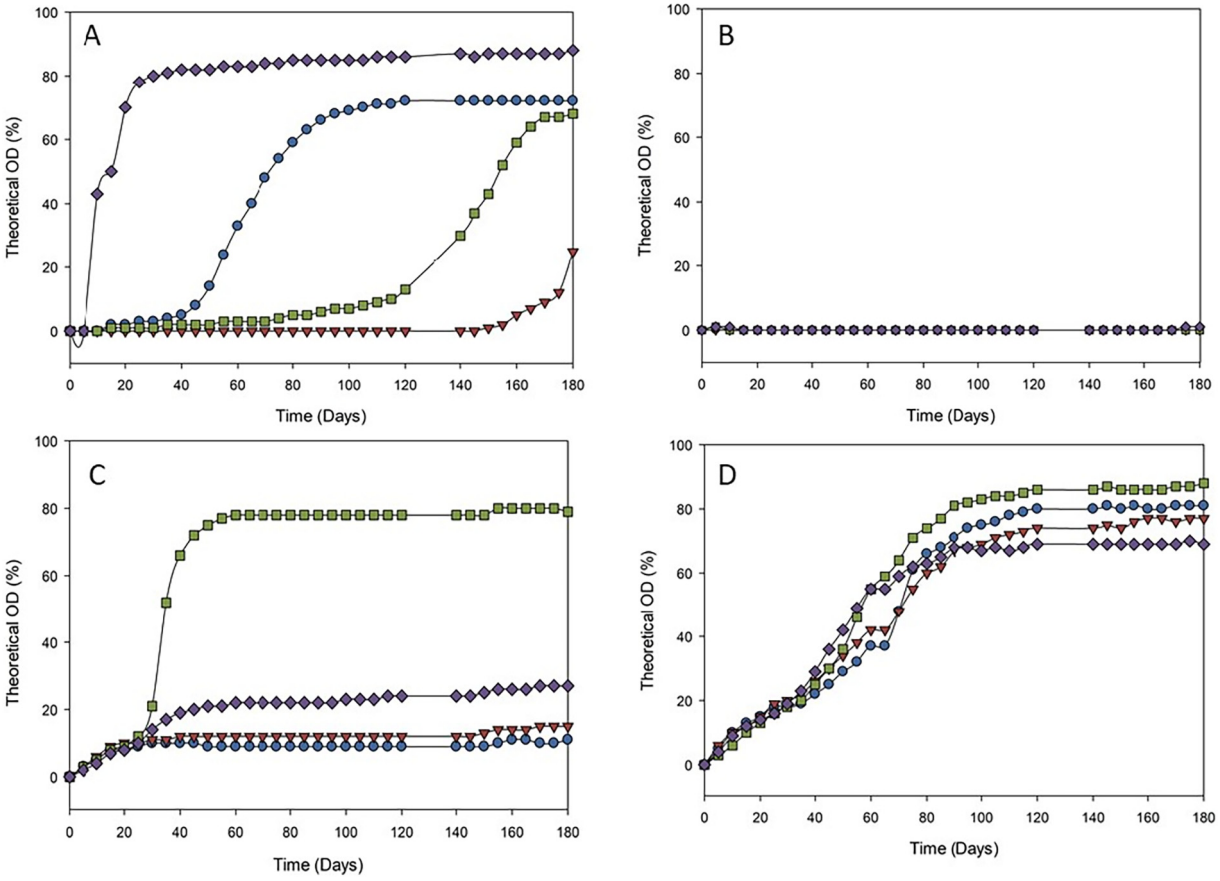

Fig. 4. Biodegradation of water-soluble polymers in river water using MR as media and inoculum. Presented as test vessel replicates from a single test. A) PEG 4000 B) PEG 35,000 C) PVOH79 D) CMC 0.6.

compound that is utilized as energy by the microbial community but the carbon that is incorporated into microbial biomass or remains in solution will not be quantified. The biomass yield (ratio of the amount of biomass produced to the amount of substrate consumed) changes depending on the test chemical, competent degrader, nutrient levels, and environmental conditions. Typical biomass yields have been measured in the lab using batch and flow through studies, estimated using stoichiometric and bioenergetic approaches, and measured in field studies. A thorough review of biomass yield estimation approaches can be found in Tchobanoglous et al. (2003). Using the stoichiometric method, Tchobanoglous et al. estimated a theoretical biomass yield of 0.39 g VSS per g COD for a representative cell structure using glucose as the food source. Tchobanoglous et al. estimated a biomass yield of 0.42 g VSS per g COD (this time using aniline as the food source) for heterotrophic bacteria under aerobic conditions using the bioenergetics approach. Tchobanoglous et al. further provided an estimate of 0.45 g VSS per g COD biomass synthesis yield for typical wastewater bacteria utilizing organic carbon under aerobic conditions. While these estimates were not made under typical biodegradation test conditions, they do show that when following respirometric endpoints (O_2 and CO_2) one would not expect a fully biodegradable compound to ever reach 100 % $ThCO_2$ or ThOD instead it is more likely that extents will reach 50–90 % depending on test set up, test substance being evaluated, and test duration. Indeed, in an OECD 301 ring test (Painter and King, 1985) that used DOC removal to confirm oxygen uptake results 64–115 % ThOD was measured for aniline biodegradation and corresponded to 96–99 % DOC removal.

The combination of multiple analytical endpoints to evaluate mineralization provides valuable insight when nonspecific analytical techniques are being leveraged. Results from ready and inherent test systems have variability from test setup impacting mass transfer rate to the detector and the resulting observed rates of mineralization, natural inoculum variability, and analytical endpoints. At times, this variability can lead to confusion in data analysis. For example, in the MR screening study 3 of the 4 PEG 4000 replicates mineralized extensively reaching 66–88 % ThOD (22 % difference) and the remaining DOC for each replicate was 3 or 4 % (indistinguishable), Table 1. Evaluation of only the ThOD endpoint may lead one to conclude that complete mineralization of the test compound did not occur for all replicates but when combined with negligible DOC remaining it is clear this is analytical endpoint variability and not actual differences in biodegradation extent.

3.5. Comparison of OECD 301B and 302B results

A subset of PEG and PEO materials (Mw = 4000; 35,000; 50,000; and 100,000 Da), the PVOH materials, and the CMC materials were evaluated in both the OECD 301B and 302B to evaluate the effect of improved ratio of microbial abundance to test chemical concentration and likely higher microbial diversity in the 302B. Disregarding test time frame, the final extent of biodegradation measured in the OECD 301B and OECD 302B closely replicated each other. PEGs and PEOs completely mineralized in both the 301B and in the 302B leaving negligible DOC. DOC removal for that class of polymers was >93 % and >96 %, in the 301B and 302B respectively, with >80 % $ThCO_2$ detected in the 301B. Similarly, mineralization of all PVOH materials reached >88 % $ThCO_2$ in both the 301B and 302B, corresponding to >96 % and >95 % DOC removal, respectively. CMC 0.6 and 0.79 degraded to similar relative extents in the OECD 301B and 302B. CMC 1.2 did not degrade in either test.

The improved biomass ratio of the OECD 302B set up resulted in faster rates of biodegradation in these test systems. However, the improvement was more dramatic for slower degrading materials. Fast degraders such as

PEG 4000, PEG 35,000 and the PVOH materials, that reached the plateau phase of degradation within 28 days in the OECD 301B, took 8–20 d to reach 60 % biodegradation in these studies and the improved biomass ratio in the OECD 302B shortened the time it took to reach 60 % degradation to 3–13 d. It had no effect of the time to 10 % as the lag phase remained 4–9 d. However, the slower degrading polymers showed a bigger improvement in rate and thus shortened test duration. PEO 50,000 and PEO 100,000 took 51 and 83 d to reach 60 % $ThCO_2$ in the OECD 301B and only 33 d in the OECD 302B. While the OECD 301B was extended to 160 d to resolve replicated variability for PEO 100,000, this variability was resolved by 40 d in the OECD 302B with both replicates reaching >95 % DOC removal. CMC 0.6 took 91 d to reach 60 % in the OECD 301B and only 30 d in the OECD 302B. The lag time to 10 % also improved from 18 to 6 days with the increase in inoculum biomass. However, it reached 88 % DOC removal in the 302B studies and 89 % in the 301B study in similar timeframes. The CMC 0.79 reached 34–39 % DOC removal in the OECD 302B and 60 % DOC removal in the OECD 301B. This is the only case where the 301B showed improved biodegradation over the 302B.

Application of OECD 302B biomass to test material ratios should be considered in the evaluation of biodegradation using CO_2 production as an endpoint. It shows consistent results compared to the OECD 301B but in a shorter timeframe with comparative analytical signal to noise. In the OECD 301B, the validity criteria state that normal blank respiration should not exceed 40 mg CO_2/L at 28 days. This is to ensure the biodegradation signal from the dosed carbon (10–20 mg C/L or 37–73 mg CO_2/L) is sufficiently distinguishable from the background respiration. The blank background respiration at 28 days in the OECD 302B studies following CO_2 production were 183 and 208 mg CO_2/L. Compared to a dose signal of 50 mg C/L or 183 mg CO_2/L, the OECD 302B test set up and inoculum treatment used in this research results in a similar signal to noise ratio compared to the OECD 301B guideline.

3.6. Regulatory assessment

Much like surfactants and oils, polymers are mixtures of structurally similar components which are expected to degrade sequentially, therefore when evaluating polymer biodegradation in an OECD 301 the 10-day window is not applicable (OECD, 2006). Applying the OECD 301B pass criteria (60 % $ThCO_2$ in 28 d (OECD, 1992)) all PEGs and PVOHs, as well as PEO 30,000 would be classified as ready biodegradable. Applying the eRBT persistence criteria of 60 % $ThCO_2$ production in 60 d (ECHA, 2017), then PEO 50,000 and PEO 300,000 would also be considered not persistent. When evaluating data from the OECD 302B study no additional materials would be considered not persistent under REACH criteria (reaching 70 % DOC removal in 7 d) and only PEG 4000 would meet the inherently biodegradable criteria, the other polymers which meet ready or enhanced ready criteria do not meet the inherent pass criteria. This observation leads to questions concerning the value of the inherent assessment from a REACH perspective as it is not accurately assessing persistence for the soluble polymers evaluated in this study. This research has shown significant value from increasing inoculum to levels used in the OECD 302B test system in terms of decreasing time to evaluate biodegradation. We would recommend that more value is put on studies using higher inoculum levels in order to decrease test system variability and decrease time to reach complete mineralization. In addition, ready and inherent biodegradation studies should be extended beyond traditional test durations until mineralization has plateaued in order to assess extent of mineralization and this data should be used by regulators in persistence assessments.

Simulation studies would provide the most environmentally realistic test material to inoculum ratios, however appropriate radiolabelled polymers are difficult to synthesize (in some cases technical knowledge does not exist yet) and correspondingly representatives of these polymer classes could not be obtained. Four major synthesis houses were contacted and only [1,2 ^{14}C]-PEG 4000 and [methyl-^{14}C] CMC were available. Upon further clarification, the methyl label on the CMC was a methylation of the carboxyl group. Thus, both available radiolabels were the most labile portion of the polymer and not appropriate for evaluation of complete mineralization but [1,2 ^{14}C] PEG 4000 could still be useful to understand primary rates of biodegradation. Custom synthesis of a randomly labelled PEG or CMC on the cellulose backbone was not available due to synthesis pathways. The issues obtaining radiolabelled polymers and technical hurdles for analytical methods to quantify parent and metabolites further highlight the need for technically robust methods leveraging nonspecific analytical endpoints such as those evaluated in this research.

4. Conclusions

As with small MW chemicals, ready and inherent tests have a significant role to play in evaluating the biodegradability of water-soluble polymers disposed down the drain. These tests are more readily available at contract laboratories, faster to conduct, with less analytical hurdles, and are less cost prohibitive than simulation studies. Some polymers with high MW and different structural components (for example, the addition of high levels of carboxy methyl substitution) will take longer to reach mineralization plateaus in ready and inherent tests and test extension should be employed to evaluate full extent of mineralization potential. It is critical that we continue to evolve test methods to better predict actual environmental conditions utilizing best available analytical techniques but still maintaining a level of practicality that allows for fast screening to aid innovation in this space. Combining information from the respirometric endpoints with dissolved organic carbon analysis at study completion provides a clearer indication of extent of mineralization. Increasing microbial density leads to decreased time to reach complete mineralization and increased reproducibility of test results. As has been observed historically for small MW molecules, as environmental realism with respect to test chemical to biomass ratios increases, then the rate of biodegradation in the study increases. Due to variability in the presence of competent degraders in small volume grab samples of river water, significant work is needed to develop a robust screening method for assessing polymer biodegradation perhaps utilizing concentration techniques to increase microbial abundance and diversity in the studies.

CRediT authorship contribution statement

Jennifer Menzies: conceptualization, methodology, data curation, original draft. **Ashley Wilcox**: methodology, investigation, data curation, visualization, writing-review and editing. **Kenneth Casteel**: methodology, investigation, data curation, visualization. **Kathleen McDonough**: conceptualization, data curation, writing-review and editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160006.

References

Beran, E., Hull, S., Steininger, M., 2013. The relationship between the chemical structure of Poly(alkylene glycol)s and their aerobic biodegradability in an aqueous environment. J. Polym. Environ. 21, 172–180.

Bernhard, M., Eubeler, J.P., Zok, S., Knepper, T.P., 2008. Aerobic biodegradation of polyethylene glycols of different molecular weights in wastewater and seawater. Water Res. 42, 4791–4801.

Boethling, R., Howard, P.H., Beauman, J.A., Larosche, M.E., 1995. Factors for intermedia extrapolation in biodegradability assessment. Chemosphere 30 (4), 741–752.

Chiellini, E., Corti, A., Salvatore, D., Solaro, R., 2003. Biodegradation of poly (vinyl alcohol) based materials. Prog. Polym. Sci. 28 (6), 963–1014.

ECHA, 2003. Technical Guidance Document on Risk Assessment: Part II Chapter 3 Environmental Risk Assessment.

ECHA, 2014. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint Specific Guidance.

ECHA, 2017. Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT/vPvB Assessment, Version 3.0.

Federle, T.W., Itrich, N.R., 2006. Fate of free and linear alcohol-ethoxylate-derived fatty alcohols in activated sludge. Ecotoxicol. Environ. Saf. 64 (1), 30–41.

Gartiser, S., Brunswik-Titze, A., Flach, F., Junker, T., Sattler, D., Johncke, U., 2022. Enhanced ready biodegradability screening tests for the evaluation of potential PBT substances. Sci. Total Environ. 833, 155134.

Haschke, H., Tomak, I., Adreas, K., 1998. Systematic investigations on the biological degradability of packing material II. On the biodegradability of polyvinylalcohol based films Systematische untersuchungen zur biologischen abbaubarkeit von verpackungsmaterial, 2. Mitt. Zur biologischen abbaubarkeit von auf polyvinylalkohol basierenden verpackungsfolien. Monatshefte fur Chemie 129 (4), 365–386.

Herzberger, J., Niederer, K., Pohlit, H., Seiwert, J., Worm, M., Wurm, F.R., Frey, H., 2016. Polymerization of ethylene oxide, propylene oxide, and other alkylene oxides: synthesis, novel polymer architectures, and bioconjugation. Chem. Rev. 116, 2170–2243.

ISO, 2018. ISO 8199 Water Quality- General requirements and guidance for microbiological examinations by culture. Third Edition.

Kawai, F., Yamanaka, H., 1986. Biodegradation of polyethylene glycol by symbiotic mixed culture (obligate mutualism). Arch. Microbiol. 146, 125–129.

Kowalczyk, A., Martin, T.J., Price, O.R., Snape, J.R., van Egmond, R.A., Finnegan, C.J., Schafer, H., Davenport, R.J., Bending, G.D., 2015. Refinement of biodegradation tests methodologies and the proposedutility of new microbial ecology techniques. Ecotoxicol. Environ. Saf. 111, 9–22.

Martin, T.J., Goodhead, A.K., Snape, J., Davenport, R.J., 2018. Improving the ecological relevance of aquatic bacterial communities in biodegradability screening assessments. Sci. Total Environ. 627, 1552–1559.

Martin, T.J., Snape, J., Bartram, A., Robson, A., Acharya, K., Davenport, R.J., 2017. Environmentally relevant inoculum concentrations improve the reliability of persistent assessments in biodegradation screening tests. Environ. Sci. Technol. 51, 3065–3073.

McDonough, K., Itrich, N.R., Casteel, K., Menzies, J.Z., Williams, T., Kravos, K., Price, J., 2017. Assessing the biodegradability of microparticles disposed down the drain. Chemosphere 175, 452–458.

OECD, 1992. Organisation for Economic Co-operation and Development. OECD 302B Zahn-Wellens/EMPA Test. OECD Guidelines for the Testing of Chemicals, Section 3.

OECD, 1992. Organisation for economic co-operation and development. OECD 306: Biodegradability in Seawater. OECD Guidelines for the Testing of Chemicals, Section 3.

OECD, 1992. Organization for economic co-operation and development. OECD 301 Ready Biodegradability. OECD Guidelines for the Testing of Chemicals, Section 3.

OECD, 2006. Organisation for economic cooperation and development. Revised Introduction OECD Guidelines for the Testing of Chemicals, Section 3.

OECD, 2014. Organisation for economic co-operation and development. OECD 310: Ready Biodegradability - CO2 in Sealed Vessels (Headspace Test). OECD Guidelines for the Testing of Chemicals, Section 3.

Painter, H.A., King, E.F., 1985. A respirometric method for assessment of ready biodegradability: results of a ring test. Ecotoxicol. Environ. Saf. 9, 6–16.

Saake, B., Horner, S., Puls, J., 1998. Progress in the enzymatic hydrolysis of cellulose derivatives. In: Glasser, H.A. (Ed.), Cellulose Derivatives. American Chemical Society, pp. 201–216.

Solaro, R., Corti, A., Chiellini, E., 2000. Biodegradation of poly(vinyl alcohol) with different molecular weights and degree of hydrolysis. Polym. Adv. Technol. 11 (8–12), 873–878.

Sparham, C., Rehman, N., Melling, J., van Duynhoven, J., Marshall, S., 2008. Biodegradability of highly ethoxylated nonionic surfactants: determination of intermediates and pathways of biodegradation. Environ. Toxicol. Chem. 27 (5), 1069–1076.

Struijs, J., van den Berg, R., 1995. Standardized biodegradability tests: extrapolation to aerobic environments. Water Res. 29 (1), 255–262.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. Metcalf and Eddy, New York, McGraw Hill.

Thouand, G., Friant, P., Bois, F., Cartier, A., Maul, A., Block, J.C., 1995. Bacterial inoculum density and probability of Para-nitrophenol biodegradability test response. Ecotoxicol. Environ. Saf. 30 (3), 274–282.

Van Ginkel, C.G., Gayton, S., 1996. The biodegradability and nontoxicity of carboxymethyl cellulose (DS 0.7) and intermediates. Environ. Toxicol. Chem. 15 (3), 270–274.

Vázquez-Rodríguez, G.A., Beltrán-Hernández, R.I., Coronel-Olivares, C., Rols, J.L., 2011. Standardization of activated sludge for biodegradation tests. Anal. Bioanal. Chem. 401 (4), 1127–1137.

West, R.J., Davis, J.W., Pottenger, L.H., Banton, M.I., Graham, C., 2007. Biodegradability relationships among propylene glycol substances in the organization for economic cooperation and development ready- and seawater biodegradability tests. Environ. Toxicol. & Chem. 26 (5), 862–871.

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Free, but not microplastic-free, drinking water from outdoor refill kiosks: A challenge and a wake-up call for urban management ☆

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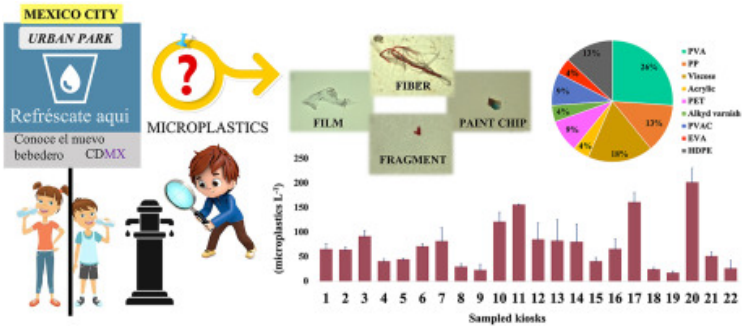
Abstract

Free refill drinking water kiosks are an essential sustainable water supply system for people in metropolitan areas worldwide. Despite their importance in urban settings, the impact of microplastic contamination remains elusive. Here, we investigated the occurrence and characteristics of microplastics in drinking-water samples collected from 22 self-distributed refill kiosks located in 14 multiuse urban parks spread across nine municipalities in Mexico City (Mexico). The results showed that microplastics were detected in all the samples, with an overall mean concentration of 74.18 ± 48.76 microplastics L^{-1}. The abundance of microplastics was significantly different between sampled kiosks, ranging from 23 ± 11.31 to 202 ± 28.39 microplastics L^{-1}. There were more fibrous microplastics (88%) than fragments (9%) and films (3%), with the majority (56%) being <200 μm in length. They were predominantly transparent (85%), with only a few being colored (15%; blue, red, green, and brown). Attenuated Total Reflection-Fourier-transform infrared spectroscopy further revealed microplastics of various polymer types, including polyvinyl alcohol, high-density polyethylene, polypropylene, polyvinyl acetate, ethylene vinyl alcohol, acrylic, alkyd resin, and viscose. Based on our findings, drinking water from urban refill kiosks exposes children more than adults to microplastics. Furthermore, the steps that should be taken at urban refill kiosks to prevent microplastic pollution while offering recreational services to people have been highlighted. Therefore, this first study serves as a wake-up call to urban water management to improve the safety of water from emerging pollutants like microplastics in the infrastructure of refill kiosks.

Graphical abstract



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Author statement

V.C. Shruti - Conceptualization, Methodology, Data curation, Writing - original draft; Gurusamy Kutralam-Munaisamy - Conceptualization, Methodology, Data curation, Writing - original draft; Fermín Pérez-Guevara - Methodology, Conceptualization; I. Elizalde Martinez – Supervision; Priyadarsi D. Roy - Methodology, Conceptualization. All authors contributed to the article and approved the submitted version....

Study area and sample collection

Mexico City is located in the central southern section of Mexico and has a population of more than 21 million people. It is comprised of 16 municipalities and requires a water supply of 32 m^3/s entering the city from basins outside of it. The implementation of a “Drinking Fountains program” is one of the key components of Mexico's water reform. The Mexican Water System issued the first federal legislation in Mexico City in 2015, establishing free drinking-water refill kiosks in...

Microplastic concentrations and morphology in outdoor drinking water kiosks

Microplastics were detected in all of the drinking water samples collected from Mexico City's kiosks located in multi-use neighborhood parks (Fig. 2a). The spatial distribution of microplastics revealed significant spatial heterogeneity. The abundance of microplastics (L^{-1}) ranged from 10 to 20 ($n = 1$), 21 to 30 ($n = 3$), 31 to 50 ($n = 4$), 51 to 100 ($n = 10$), 101 to 200 ($n = 3$), and >200 ($n = 1$) among the sampled sites, where n is the number of sampled kiosks. The abundance of microplastics (L^{-1}...

Conclusions

This study for the first time explored the occurrence and characteristics of microplastics in free drinking water collected from urban outdoor refill kiosks across Mexico City. The data shows that microplastics were detected in all the 22 refill kiosks evaluated, demonstrating the widespread presence of microplastic contamination in drinking water supplied to the locals. There was a significant variation in microplastic abundance across the sampled zones and kiosks. Microplastics of sizes...

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper....

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References (55)

J. Zhu *et al.*
Microplastics in dust from different indoor environments
Sci. Total Environ. (2022)

X.J. Zhou *et al.*
Microplastic pollution of bottled water in China
J. Water Proc. Eng. (2021)

A. Winkler *et al.*
Does mechanical stress cause microplastic release from plastic water bottles?
Water Res. (2019)

Z. Wang *et al.*
Occurrence and removal of microplastics in an advanced drinking water treatment plant (ADWTP)
Sci. Total Environ. (2020)

S. Viaroli *et al.*
Microplastics contamination of groundwater: current evidence and future perspectives. A review
Sci. Total Environ. (2022)

A. Turner
Paint particles in the marine environment: an overlooked component of microplastics
Water Res. (2021)

V. Torretta
Environmental and economic aspects of water kiosks: case study of a medium-sized Italian town
Waste Manage. (Tucson, Ariz.) (2013)

J. Sun *et al.*
Microplastics in wastewater treatment plants: detection, occurrence and removal
Water Res. (2019)

S. Soroldoni *et al.*
Are antifouling paint particles a continuous source of toxic chemicals to the marine environment?
J. Hazard Mater. (2017)

V.C. Shruti *et al.*
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